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Randomised, 2-Sequence, 4-Period Replicate Cross-Over Bioequivalence Study of A New Riluzole Orodispersible Film Vs. A Reference Tablet in Healthy Volunteers

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Abstract

Purpose: The present bioequivalence study aimed at demonstrating the bioequivalence of a recently developed novel riluzole orodispersible film vs. a reference tablet.

Methods: Healthy male and female volunteers received single oral doses of 50 mg of riluzole, as test and reference formulation, under fasting conditions, in each of 4 subsequent periods separated by wash-out intervals of at least 7 days, according to a 2-treatment, 4-period, replicate randomised cross-over design.

Findings: Riluzole plasma concentrations were almost superimposable. Riluzole attained a similar peak concentration (315.62±124.95 ng/mL with the film and 278.81±123.32 ng/mL with the tablet) at a median t_{max} of 0.75 h after both treatments. Then, riluzole plasma concentrations showed a superimposable decline from the peak up to 36 h post-dose, with mean half-lives of 10.22±1.66 and 10.22±1.48 h with the film and the tablet. Mean AUC_{0-t} was 1263.40±571.58 h*ng/mL with the film and 1135.98±514.98 h*ng/mL with the tablet. The 90% confidence intervals of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of riluzole fell within the predefined range 80.00-125.00%. The treatments did not differ significantly either in t_{max} or $t_{1/2}$. On average, the test orodispersible film dissolved on the tongue in a median time of about 2.5 min with a range of 0.7-5.7 min. Orodispersible film palatability was good or acceptable for most subjects.

Implications: Riluzole bioavailability after single dose of the test treatment was equivalent to that of the reference treatment in both rate and extent of absorption, thus fully satisfying the bioequivalence criteria. The test treatment showed a good tolerability similarly to the reference and is expected to improve the patients' compliance.
Registered at ClinicalTrials.gov with the identifier NCT04819438.

Keywords: Riluzole, Orodispersible Film, Bioequivalence, ALS, Palatability, Pharmacokinetic

List of Abbreviations

λz Terminal elimination rate constant
AE Adverse Event
ALS Amyotrophic lateral sclerosis
ANOVA Analysis of Variance
AUC_{0-t} Area under the concentration-time curve from time zero to time t
$AUC_{0-\infty}$ Area under the concentration vs. time curve up to infinity
BMI Body mass index
CI Confidence interval
C_{max} Peak drug concentration
CV Coefficient of Variation
CV_{WR} Coefficient of Variation within-subject
CYP Cytochrome P450
ECG Electrocardiogram
EMA European Medicines Agency
GCP Good Clinical Practice
ICH International Conference on Harmonisation
LC-MS/MS Liquid chromatography-tandem mass spectrometry
LQL Lower Quantification Limit
MedDRA Medical Dictionary for Regulatory Activities
min Minute
N Number of observations
PE Point Estimate
PK Pharmacokinetics
PT Preferred Term
SD Standard Deviation
SOC System Organ Class
t1/2 Half-life
tmax Time to achieve Cmaxs
µL - microliter
µm - micrometer
AUC_{extra} - Percentage of the residual area (Ct/λz) extrapolated to infinity in relation to the total AUC0-∞,
C - Celsius
h - hour
HPLC - High-performance liquid chromatography
HCl - Hydrochloric acid
Li - Lithium
g - gravity
mL - milliliter
mM - millimolar
mm - millimeter
ng - nanogram
nm - nanometer
Ph. Eur. - European Pharmacopeia
R^2 - determination coefficient
rpm - rotations per minute

QC - quality control
v/v - volume to volume

Introduction

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder characterised by rapidly progressive weakness, muscle atrophy and fasciculation, spasticity, dysarthria, dysphagia and respiratory impairment. ALS usually is progressive and fatal with most affected patients dying of respiratory insufficiency after 2 to 3 years from the onset of symptoms, although occasional individuals have a more indolent course and survive for many years [1].

Although the pathogenesis of ALS is not completely elucidated, it is suggested that glutamate (the primary excitatory neurotransmitter in the central nervous system) plays a role in cell death in the disease. Riluzole (2-amino-6-trifluoromethoxybenzothiazole, $C_8H_5F_3N_2OS$) is an anti-glutamatergic agent with neuroprotective properties that has been developed for the treatment of ALS [2-5]. Riluzole has been shown to exert neuroprotective effects and prolong survival in patients with ALS [6-9], but has no effect on the degradation of muscular function [10,11].

A recently developed riluzole orodispersible film, approved by FDA in 2019 [12-16], is expected to fill an important medical need. Indeed, oral tablets currently used to treat ALS patients can represent a challenge, if the patients' swallowing distress is borne in mind. A medication such the novel riluzole orodispersible film that can be easily administered without water may improve the quality of life for ALS patients and improve patient care [14,15]. In fact, the patient or caregiver needs only to place the film on the tongue, where it can dissolve into the saliva and be ingested with intentional swallowing or during the normal reflex of swallowing, thus eliminating the need for swallowing a tablet with liquid or crushing it into soft food [14].

The primary objective of the present bioequivalence study was to investigate the bioequivalence of the novel formulation versus commercially available 50 mg tablets. As the secondary objectives, dissolution time and palatability of the novel orodispersible film were evaluated.

Participants and Methods

Study Design

The present study design was single-center, single dose, open-label, randomised, 2-sequence, 4-period replicate cross-over. The study compared the pharmacokinetic profile of riluzole after replicate single dose of the novel orodispersible film test formulation (Aquestive Therapeutics, USA) against reference film-coated tablets to evaluate their bioequivalence in healthy men and women.

Study Population

The study was performed at the Phase I Unit of CROSS Research S.A., Arzo, Switzerland.

Healthy men and women were enrolled in this study according to the following main inclusion criteria which were standard criteria for pharmacokinetics studies, with the addition of some specific criteria due to known drug-to-riluzole interactions, namely: (i) age of 18 to 55 y, (ii) a body mass index between 18.5 and 29 kg/m^2, (iii) non-smokers for at least 6 months before the study with a negative cotinine test, (iv) good health based on medical history, physical examination, a 12-lead electrocardiogram (ECG) and routine haematology and blood chemistry tests, (v) women of child-bearing potential using at least one reliable method of contraception with exception of hormonal oral, transdermal, implanted, injected, intravaginal or intrauterine contraceptives, (vi) willingness to provide written informed consent.

Main exclusion criteria (i) intake of any medication, (ii) a history of drug or caffeine (>5 cups coffee/tea/day) abuse, (iii) history of alcohol consumption in excess of two drinks per day in men and one drink per day in women, (iv) ascertained or presumptive hypersensitivity to the active compound or history of anaphylaxis to drugs.
In particular, no medication was allowed for 2 weeks before the start of the study and during the whole study duration. Central nervous system depressants and CYP inhibitors or hormonal oral or transdermal contraceptives were forbidden for 30 days before study screening and during the whole study duration.

Implanted, injected, intravaginal or intrauterine hormonal contraceptives were forbidden for 6 months before study screening and during the whole study duration.

Paracetamol was allowed as therapeutic countermeasure according to the investigator's opinion.

Investigational Treatments

The subjects received single oral doses of 50 mg of riluzole, as test orodispersible film (Aquestive Therapeutics, USA) and reference film-coated tablets (Rilutek®, Sanofi Mautre IP, France) under fasting overnight conditions (at least 10 h), in each of 4 subsequent periods separated by wash-out intervals of at least 7 days between consecutive administrations, according to a 2-treatment, 4-period, replicate cross-over design.

Each study dosing was performed in the morning under fasting conditions.

Before each film administration, the subjects drank still mineral water to wet their mouth.

Afterwards, the investigator or deputy placed the orodispersible film directly on the dorsal aspect of the subjects' tongue. Dosing time was defined as the time the film was placed on the tongue. Saliva swallowing was allowed, but not to chew, bite, or swallow the film. Oral cavity was inspected 1-, 2- and 5-min post-dose until either complete dissolution was confirmed or until the subject alerted the study staff of the dissolution of the film.

Film-coated tablets were swallowed (without chewing) with 150 mL of still mineral water.

Dissolution Tests

Twelve (12) tablets of each test and reference product were used for evaluation of dissolution in vitro. Dissolution tests were performed in 900 mL of Ph. Eur. recommended dissolution media: 0.1N HCl, pH 4.5 buffer and pH 6.8 buffer to cover the physiological pH range, maintained at 37.0 ± 0.5° C. Apparatus 1 (baskets, 40 mesh stainless steel) with agitation speed 50 rpm was used. Samples were collected at 5, 10, 15, 20, 30 and 45 minutes and riluzole was assayed using a HPLC method with ultraviolet–visible spectrophotometer at 220-nm wavelength. The results of in vitro dissolution were reported as similarity factor (f2).

Ethical Procedures

The independent ethics committee of Canton Ticino reviewed and approved the documentation of the study and the Swiss Federal Health Authorities (Swissmedic) authorised the study in June 2020. The study was conducted in compliance with the Swiss ordinance on clinical trials of therapeutic agents and in accordance with the Declaration of Helsinki and the general principles of ICH Harmonised Tripartite Guidelines for GCP. Study subjects did not undergo any study procedure before signing the written informed consent form. The study was registered at ClinicalTrials.gov with the identifier NCT04819438.

Pharmacokinetic, Palatability and Safety Parameters

The concentration of riluzole in plasma was measured at 0 h (pre-dose), 15, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h post-dose.

The following PK parameters were measured and/or calculated for plasma riluzole, using the validated software Phoenix WinNonlin® version 6.3: Cmax and AUC0-t (primary variables) and AUC0-∞, t1/2, tmax, AUCextra, λz, (secondary variables). A linear trapezoidal method was used to calculate AUC. A non-compartmental method was applied. The quality of log-linear regression (and, consequently, the reliability of the extrapolated PK parameters) was demonstrated by a determination coefficient $R^2 \geq 0.8$.

The subjects were asked about the palatability of the test product immediately after administration. Palatability was scored as very unpleasant, unpleasant, acceptable, good or very good on a scale going from 0 (very unpleasant) to 4 (very good).

Adverse events and vital signs were recorded throughout the study. Full physical examinations were performed at the screening and at the end of the study.

Laboratory analysis including haematology, blood chemistry and urine assays was performed at the screening and the end of the study.

The investigator or deputy inspected the subjects' mouth to check for mucosal irritation at the application site after each administration of the film, at pre-dose and 0.5 and 1 h post-dose.

Sample Collection, Handling and Analytics

Blood samples (7 mL) for PK analysis were collected using an indwelling catheter with switch valve.

Blood was collected from the catheter and transferred with a syringe into pre-labelled Li-heparinised polypropylene tubes. The samples were stored on ice for a maximum of 60 min. Then the samples were centrifuged at 4° C for 10 min at 2500 g to obtain plasma. Each plasma sample was immediately divided into 3 aliquots in polypropylene tubes and stored frozen until analyses.

The concentration of riluzole in plasma was determined at Accelera S.r.l., Italy, using a fully validated LC-MS/MS method with a lower quantification limit (LQL) of 0.5 ng/mL.

A full validation of the method was performed according to the current guidelines for bioanalytical method validation. The long-term stability of riluzole in plasma was tested. Samples were stored at -80° C ± 10° C at the laboratory facilities. The LC-MS/MS method for the determination of riluzole in human plasma produced accurate and precise results. With respect to the accuracy of the method, the validation study revealed absolute biases for the Quality Control (QC) samples at the levels LQL (LQC), Low (QC-Low), Medium (QC-Medium), High (QC-High) of -6.6, 3.3, 5.3 and 1.8%, respectively. The precision results (expressed as total CV%) were as follows: 16.5, 4.2, 2.6 and 2.8%, for the LQC, QC-Low, QC-Medium, and QC-High samples, respectively.

Intra-run CVs (repeatability) were 13.6, -0.7, 0.7 and 3.0% for the LQC, QC-Low, QC-Medium, and QC-High samples, respectively. The calibration range covered 0.5 - 500 ng/mL. Mean ± SD R^2 of the calibration curves was 0.9954±0.0025.

The bioanalytical procedure foresaw that 50 µL of human plasma were added with 500 µL of acidified acetonitrile (acetonitrile + 0.5% formic acid) containing 10 ng/mL of internal standard (riluzole-^{13}C, $^{15}N_2$). Samples were then centrifuged. The supernatant was transferred and dried under nitrogen stream at 40° C. Extracted samples were resuspended with 200 µL of 10 mM ammonium formate + 0.1% formic acid: acetonitrile (1/1, v/v). Samples of 10 µL were injected in the HPLC system *via* 5 µL loop. A Kinetex 2.6

μm C18 column (50 x 2.1 mm) was used to perform the chromatographic analysis under gradient conditions. Mobile phase A was 10 mM ammonium formate containing 0.1% formic acid and mobile phase B was acetonitrile. Retention times of riluzole and internal standard are typically 2.4 min. Detection was operated in positive ion mode.

Data Analysis

Study data were described using classic descriptive statistics for quantitative variables and frequencies for qualitative variables. The statistical analysis of demography and safety data was performed using SAS® version 9.3 (TS1M1). The statistical analysis of pharmacokinetic parameters was performed using Phoenix WinNonlin® version 6.3 and SAS® version 9.3 (TS1M1). C_{max} and AUC were compared using analysis of variance (ANOVA) for a 2-sequence, 4-period replicate cross-over design on log-transformed data. Acceptance criterion for bioequivalence was that the 90% confidence interval (CI) for the test/reference ratio of C_{max}, $AUC_{0-\infty}$ and AUC_{0-t} geometric means was within the 80.00-125.00 range, according to the current EMA guideline for bioequivalence investigations.

The well-known high variability of riluzole led to the choice of the replicate cross-over design as suggested by the current EMA guideline. According to the guideline, an enlargement of the acceptance interval for bioequivalence in terms of C_{max} is allowed up to 69.84-143.19% in relation to the actual intra-subject variability of riluzole C_{max} found in the study with the reference treatment, by keeping in consideration the favourable safety profile of the test formulation previously observed in 3 clinical studies.

Sample Size Calculation

Variability (CV_{WR}) of AUC_{0-t} (0.1265 with a δ of 1.0917) and C_{max} (0.3266 with a δ of 1.11) observed in a previous study was used in the calculation. The actual ratio of geometric means in the previous study was 1.0917 for AUC_{0-t} and 1.1582 for C_{max}. The calculation of the sample size for the present study using a δ of 1.1582 would be 126 subjects. Considering that the enrolment of at least 126 subjects in a bioequivalence study would be hardly justifiable and that the actual δ of C_{max} obtained in another previous pilot study was 0.9436, which is notably nearer the equality, i.e., ratio = 1.00, than the ratio 1.1582 observed later, a ratio of 1.11 was postulated in the present sample size calculation. Indeed, maintaining CV_{WR}, α and β unchanged and assuming as C_{max} ratios, alternately, 1.05 and 1.10, sample sizes of 26 and 46 subjects, respectively, would be necessary to demonstrate bioequivalence in terms of C_{max}.

Taking into account this premise, when the sample size in each sequence group is 26 (and the total sample size is 52), a replicate crossover design would have 80% power to demonstrate bioequivalence assuming that each t-test is made at the 5.0% significance level.

In conclusion, 54 subjects were enrolled in order to have 52 completed subjects.

Randomisation And Blinding

The study subjects were assigned to one of 2 sequences of treatments (either RTRT or TRTR) according to their randomisation number. Randomisation number was given to the subjects on study Day -1, period 1, as soon as they were enrolled in the study. Balance between the sequences was made so that subjects had the same chances to be assigned to either sequence (either RTRT or TRTR in the 4 consecutive periods).

The randomisation list was computer-generated using the PLAN procedure of the SAS® version 9.3 (TS1M1). The randomisation list was supplied to the Phase I Unit and to the manufacturer for the preparation of the study drug individual kits before study start.

This was an open trial and no masking procedure was applied. An open-label design was used since the primary endpoint of the study is based on objective measurements of riluzole in plasma and the outcome variables could not be influenced by the subjects or investigator being aware of the administered products. The bioanalysis was performed under blinded conditions.

Results

Comparative Dissolution Study

Dissolution tests of the test orodispersible film and reference film-coated tablets performed in 0.1N HCl dissolution media showed complete riluzole release. The similarity factors (f2) were 51 – 54 which are within the f2 acceptance criteria of 50 – 100. Therefore, f2 indicated similarity. The dissolution profiles in pH 4.5 and 6.8 buffers were incomplete in 45 min. At pH 4.5, the proportion of released riluzole was 41% and 33% from the tablet and the film, respectively. pH 6.8 buffer dissolution medium showed 46% and 51% riluzole released from the tablet and the film, respectively. The incomplete dissolution is consistent with the lower equilibrium solubility of riluzole at higher pH like 4.5 and 6.8 and the presence of an excipient designed to limit riluzole release from the film at higher pH.

Disposition Of Subjects and Demography Data

The first subject was enrolled on 15JAN21 and the last subject completed the trial on 14MAR21. The disposition of subjects is depicted in Figure 1. Fifty-three (53) of the 54 enrolled subjects received at least one dose of reference and were included in the safety analysis, while all 51 completers received both planned doses of treatments and were included in the PK analysis. Demographic data (mean, median and frequency data) are presented in Table 1.

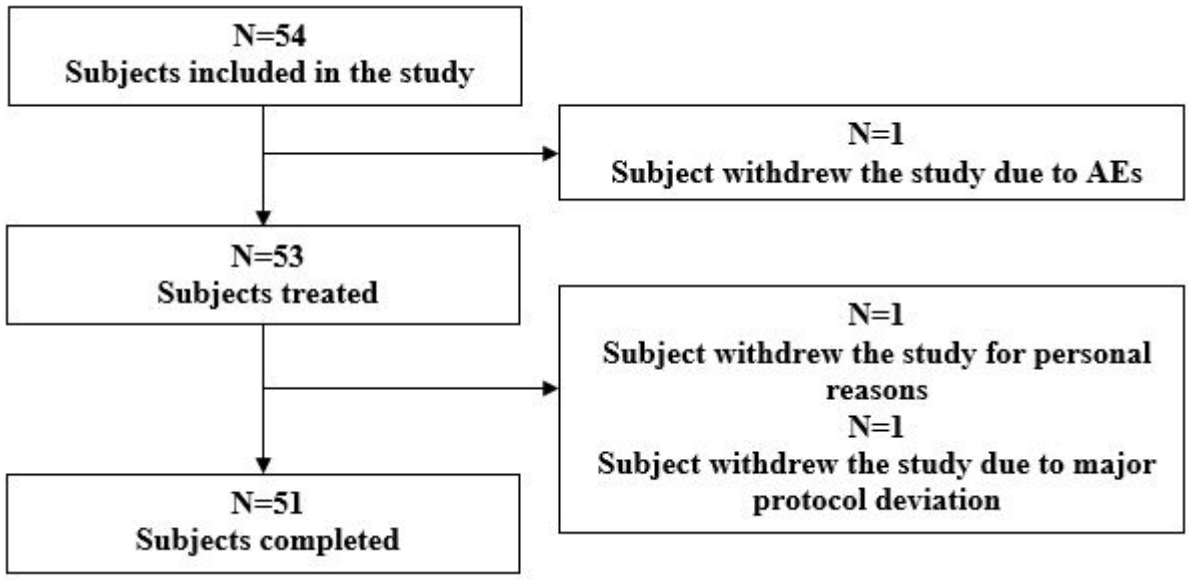

Figure 1: Disposition of Subjects

Demographic data	Enrolled set - N=54
Sex	
Women – n (%)	33 (61.1)
Men – n (%)	21 (38.9)
Age (years)	
Mean ± SD	40.3±10.2
Median (range)	41.0 (18 – 55)
Body weight (kg)	
Mean ± SD	65.86±10.87
Range	64.95 (45.8 – 90.3)

Height (cm)	
Mean ± SD	167.8±8.9
Median (range)	168.0 (150 – 190)
BMI (kg/m²)	
Mean ± SD	23.30±2.65
Median (range)	23.00 (18.7 – 28.6)
Race	
Asian – n (%)	1 (1.9)
White – n (%)	51 (94.4)
Mulatto – n (%)	2 (3.7)

N: observations; n (%): number of subjects and percentage; SD: standard deviation

Table 1: Demographic and other baseline data

Pharmacokinetic Data

Riluzole plasma concentrations were almost superimposable after replicate single dose of test and reference formulation as shown by the mean curves in Figure 2. Riluzole attained a similar peak concentration (C_{max}) at a median t_{max} of 0.75 h after both treatments, though the peak was slightly lower after the administration of tablets than film. Then, riluzole plasma concentrations after the test and after the reference treatment showed a superimposable decline from the peak up to 36 h post-dose.

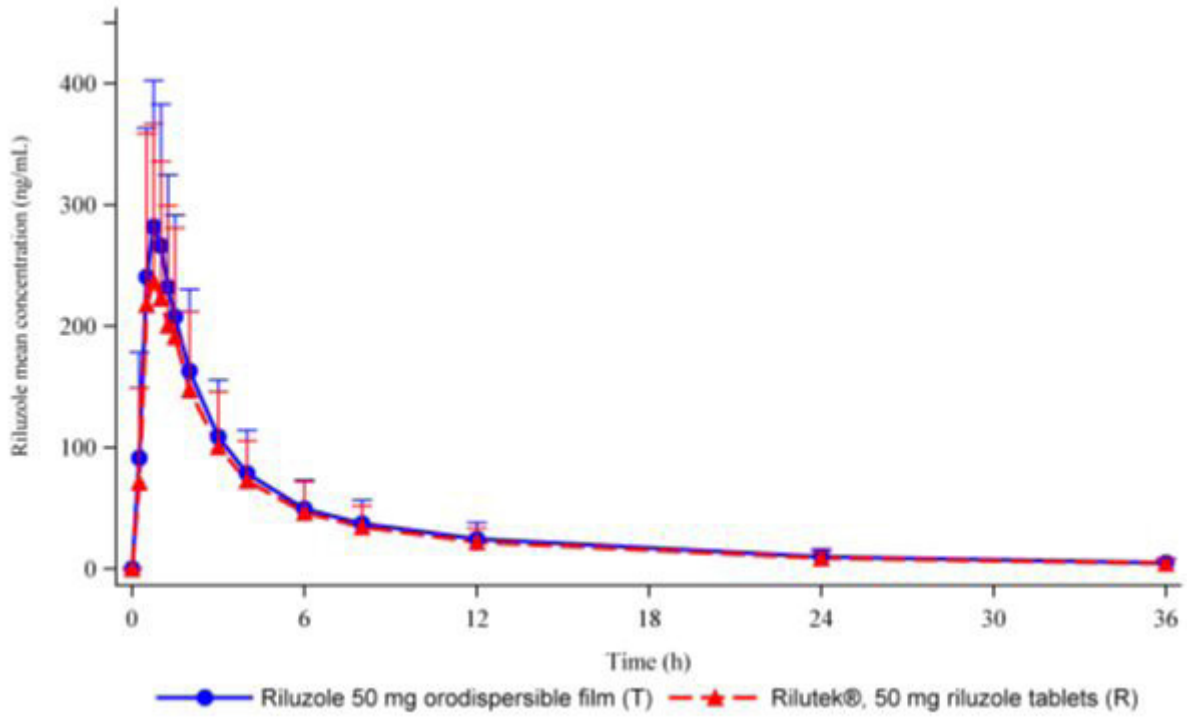

Figure 2: Mean (+SD) plasma riluzole concentrations (ng/mL) vs. time profiles after single dose of test and reference. Linear scale (N=102)

Main pharmacokinetic parameters of plasma riluzole are presented in Table 2.

On average, riluzole plasma concentrations showed similar AUC after replicate single dose of test and reference formulation, though both AUC_{0-t} and $AUC_{0-\infty}$ were on average slightly lower after single dose of the reference.

Treatment	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	AUC_{extra} (%)	$t_{1/2}$ (h)
Film	*315.62±124.95*	*0.75 (0.25-2.00)*	*1263.40±571.58*	*1348.31±630.80**	*5.42±2.25*	*10.22±1.66**
Tablet	*278.81±123.32*	*0.75 (0.25-4.00)*	*1135.98±514.98*	*1207.79±566.13*	*5.45±2.11*	*10.22±1.48*

**: N=101; mean±SD is reported except for t_{max} for which median (range) is shown*

Table 2: Pharmacokinetic parameters of plasma riluzole measured and calculated after replicate single dose of test and reference formulation (N=102)

The outcome of the statistical comparisons of the riluzole PK parameters between test and reference formulation is summarised in Table 3. The bioequivalence of the tested treatments was proven both in terms of C_{max} and AUC of plasma riluzole whose CIs were included in the 80.00-125.00% acceptance interval.

Comparison	Parameter	PE (%)	90% CIs (%)
Film vs. tablet	**AUC_{0-t}**	111.82	108.25-115.50
	$AUC_{0-\infty}$	111.83*	108.19-115.29*
	C_{max}	117.05	110.43-124.06

**: N=101;*

Table 3: Outcome of the statistical comparisons between test and reference formulation on C_{max}, $AUC_{0-\infty}$ and AUC_{0-t} of plasma riluzole. The point estimate (PE) and the 90% CIs are shown (N=102)

Film Dissolution Time

At the 1st dosing of the film, the median time of dissolution was 2.63 min with a range of 0.7-5.7 min, while, at the 2nd dosing, it dissolved in a median time of 2.45 min with a range of 0.8-5.1 min. No film was accidentally swallowed within 5 min of dosing, but one subject accidentally swallowed a part of the film 20 sec after dosing. The occurrence was reported as a minor protocol deviation.

Palatability

Contingency table of palatability evaluation by the subjects is presented in Table 4.

	Safety Set	
Palatability score	**orodispersible film 1st administration N=52**	**orodispersible film 2nd administration N=51**
0 - very unpleasant	0 (0.0)	0 (0.0)
1 - unpleasant	1 (1.9)	2 (3.9)
2 - acceptable	15 (28.8)	14 (27.5)
3 - good	31 (59.6)	28 (54.9)
4 - very good	5 (9.6)	7 (13.7)

N: observations; number and percentage of subjects are shown

Table 4: Contingency tables of palatability - Safety set

The majority of subjects evaluated the film palatability as good at both administrations, namely 59.6% at the 1st dosing and 54.9% at the 2nd dosing. The second most frequently chosen evaluation was acceptable at a frequency of 28.8% after the 1st dosing and 27.5% after the 2nd dosing. The palatability was evaluated as unpleasant at a very low frequency, namely 1.9% after the 1st dosing and 3.9% after the 2nd dosing.

Safety Data

The frequency of the adverse events is reported in **Table 5**.

MedDRA description SOC and PT term	**Film N=52**		**Tablet N=53**	
	AEs n	**Subjects n (%)**	**AEs n**	**Subjects n (%)**
Total number of AEs and of subjects with at least one AE	**122**	**52 (100)**	**19**	**14 (26.4)**
Gastrointestinal disorders	**105**	**52 (100)**	**3**	**2 (3.8)**
Hypoaesthesia oral	102	52 (100)	0	0
Dyspepsia	1	1 (1.9)	1	1 (1.9)
Nausea	1	1 (1.9)	1	1 (1.9)
Vomiting	1	1 (1.9)	1	1 (1.9)
Nervous system disorders	**17**	**12 (23.1)**	**15**	**14 (26.4)**
Headache	15	11 (21.2)	15	14 (26.4)
Dizziness	1	1 (1.9)	0	0
Presyncope	1	1 (1.9)	0	0
Musculoskeletal and connective tissue disorders	**0**	**0**	**1**	**1 (1.9)**
Neck pain	0	0	1	1 (1.9)

MedDRA version 24.0

Table 5: Number of subjects reporting and number of reported adverse events by treatment, system organ class (SOC) and preferred term (PT) (Safety set)

All 53 treated subjects (100%) experienced at least one adverse event and at least one treatment-related adverse event. The frequency of overall events was 100% with the orodispersible film and 26.4% with the tablet. Overall, 141 events occurred in the study, the investigator judged 133 of which as related to the treatment.

The most frequently reported event was oral hypaesthesia (verbatim: feeling of oral hypaesthesia), which occurred with the film only, and was of mild intensity and transient. The second most frequent event was headache which occurred at a similar frequency after tablet and film administration. All events resolved spontaneously. No relevant effects of the study treatment on blood pressure, heart rate, body weight, mouth conditions or laboratory parameters were observed.

No serious adverse event occurred during the study. No subject discontinued the study due to safety reasons.

Discussion

The present study demonstrated the bioequivalence of the novel riluzole orodispersible film vs. the reference tablet formulation in 51 healthy male and female subjects who received replicate single doses of both formulations in 4 consecutive periods separated by actual wash-out intervals of 9-12 days.

The kinetic profiles of riluzole after replicate single dose of the two treatments were compared and were found to be similar in rate and extent of absorption. In compliance with the European guideline on bioequivalence studies, the test treatment fully satisfied the bioequivalence criteria as compared to the reference treatment, both when C_{max} and AUCs were compared using the acceptance reference interval 80.00-125.00%. Indeed, no widened acceptance interval was necessary because the within-subject variability of C_{max} after reference administration was not >30%; namely, CV_{WR}% was 27.9%, which did not confirm literature data about a high riluzole within-subject variability. Furthermore, the treatments did not differ significantly either in t_{max} or $t_{1/2}$.

The test treatment showed good tolerability similarly to the reference treatment. The transient episodes of mild oral hypaesthesia occurring after administration of the film are a known side effect: riluzole, in fact, has intrinsic anaesthetic properties due to partial blocking of sodium channels [17,18]. However, such untoward effect resolves spontaneously and rapidly and does not represent any critical safety concern with the therapeutic use of the riluzole film. With respect to the remaining reported adverse events, most headache episodes occurred with the film were mild in severity, whereas headache episodes with the tablet were mostly moderate in intensity.

Notably, no liver function test elevation was observed in the study, while 100-mg riluzole daily doses (50 mg twice daily) are associated with increased rates of alanine aminotransferase (3), although the present study tested four single doses.

About one-third of ALS patients show a bulbar onset with dysphagia and dysarthria. Yet, independent of the clinical onset, dysphagia emerges in more than 80% of patients during the advanced phases of the disease [14,19,20]. Taking into account this premise, medications available only in solid dosage forms do not represent an optimal approach to the patient's treatment and care, because the inability to swallow a tablet may result in poor treatment adherence and early treatment discontinuation [12,14]. Literature studies reported that riluzole tablets have been often crushed and dispensed with food to avoid the difficulty in swallowing the solid dosage from [14,19]. No data are available to attest that the efficacy and safety of crushed riluzole tablets can be assumed the same as intact tablets. Furthermore, dysphagic ALS patients can suffer from a larynx sensory deficit which increases the risk of involuntary aspiration of the buccal content. Under these conditions, the practice of crushing riluzole tablets could impairs the swallowing ability [14,19]. In addition, when crushed riluzole tablets are dispersed in food, the conditions of larynx sensory deficit could prolong the potential anesthetic effect of riluzole, also considering that ALS patients need longer to complete a meal [19]. Starting from 2015, a riluzole oral suspension has been available to ALS patients [14,19,21,22]. The introduction of the oral suspension improved the administration mode of riluzole and the compliance of ALS patients with

dysphagia since it allowed avoiding crushing tablets and dispersing them in food, practices that are not in line with a safe and effective use of riluzole [14, 19]. However, the oral suspension did not prove to be bioequivalent to riluzole tablets differently from the novel riluzole orodispersible film which proved to be fully bioequivalent to the 50 mg riluzole tablets in the present study. In fact, the bioequivalence study of the oral suspension showed an equivalent extent of exposure to riluzole, with a ratio of 106.84% and a 90% confidence interval 96.98-117.71% matching the bioequivalence acceptance interval 80-125.00%, whereas the two formulations were not bioequivalent in terms of rate of absorption with a ratio 122.32% and 90% confidence interval 103.28-144.88%, which did not match the acceptance interval 80.00-125.00% [21]. With the oral suspension, riluzole C_{max} is approximately 20% higher than with the 50 mg tablet [21], whereas C_{max} was fully bioequivalent in the present study between the novel orodispersible film and the reference tablet with a ratio of 117.05% and a 90% confidence interval 110.43-124.06%, matching the acceptance interval 80.00-125.00%. The novel orodispersible film not only proved to be fully bioequivalent to the reference tablet, when compared to the oral suspension, but also showed a good palatability, differently from the oral suspension, whose palatability was recently studied [23]. Indeed, film palatability has been recognized as a crucial property in the recent literature [16]. In detail, the flavour of riluzole oral suspension was rated as unpleasant by 53.8% of the treated patients and strongly unpleasant by the majority of them [23]. Also, the oral suspension consistency was rated negatively, as the 19.2% of patients found it as unpleasant [23]. On the contrary, the palatability of the film was evaluated as good by the 55-60% of the subject and as acceptable by another 28-29%. No subject defined the film as very unpleasant. Considering that the film dimensions are 32.0x22.0 mm with an irrelevant height and that it dissolves in approximately 2.5 min, any effect of the film consistency can be considered as negligible. Palatability, physical dosage form and dissolution time of the test novel orodispersible films, in addition to the proven bioequivalence to the reference tablets, are expected to improve the safety of and increase the compliance and adherence to treatment with riluzole also considering, as reported from the recent literature [12], that the adherence to the treatment with riluzole tablets and oral suspension is reported as low by 55.6% and 44.4% of the patients, respectively [23].

Conclusions

The newly developed 50 mg riluzole formulation incorporating the substance into a proprietary polymer-based orodispersible film matrix proved to be bioequivalent to a reference 50 mg tablet, in both rate (C_{max}) and extent (AUC) of systemic absorption of riluzole measured in healthy male and female subjects in a replicate 4-period cross-over study. In addition, no significant difference between treatments was found either in riluzole t_{max} or $t_{1/2}$. The test treatment showed a safety profile similar to the reference treatment.

On the basis of the physical and pharmaceutical properties of the novel film and taking advantage from the proven good palatability and quick dissolution time, the test formulation is expected to fill an important medical need in patients with ALS thanks to a more user-friendly and comfortable conformation.

Author Contributions

The Sponsor, Zambon S.p.A., reviewed and approved the study design. M.M.R. reviewed and approved the study design, was responsible for the clinical activities and collected the data, A.S. and A.G. performed the analyses of pharmacokinetic and safety data and A.G. was responsible for the activities of data management and biometry, C.B. was responsible for the bioanalysis of riluzole, I.M. and C.C. reviewed and approved the design of the study and the draft manuscript and A.F.D.D. wrote the clinical study protocol, the clinical trial report and drafted the manuscript. All authors read and approved the manuscript.

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Disclosure Of Conflicts Ofof Interest

All the authors had potential conflicts of interest.

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