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Research article

Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for Analysis of Riluzole in Human Plasma and Its Application on a Bioequivalence Study

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Abstract

Introduction. As the first approved drug for amyotrophic lateral sclerosis (ALS) treatment, riluzole is known as a glutamatergic neurotransmission inhibitor administrated in 50 mg tablets twice daily. For this reason, a generic product of riluzole has been developed at a lower price by Hogar-Daroo, Iran, which would benefit patients.

Aim. The objective of this study is to develop and validate a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the analysis of riluzole in human plasma samples and its application in the bioequivalence study of riluzole tablet.

Materials and methods. The chromatography was performed by using a C18 column (100 mm, 4.6 mm, 5 mm), 0.1 % formic acid and acetonitrile (60 : 40, v/v) as the mobile phase, at a flow rate of 0.90 ml/min in the gradient program. Carbamazepine was used as an internal standard (IS). The method employed only 100 µL of human plasma for quantification by a liquid-liquid extraction technique. The multiple reaction monitoring modes (MRM) was used for quantification of ion transitions m/z 235.0/165.9 and m/z 137.6/110.0 for riluzole and the m/z 236.9/194.0 for the IS. Dwell time was set at 200 ms.

Results and discussion. The calibration curve was linear over the concentration range 0.5–300 ng/mL. The lower limit of quantitation (LLOQ) was obtained at 0.5 ng/mL. The intra-day and inter-day accuracy ranged from 93.21 % to 101.34 % and 91.77 % to 104.88 % respectively. The intra-day and inter-day precision values ranged from 2.19 % to 5.69 % and 1.67 % to 5.31 % respectively, all within the FDA acceptable ±15 %.

Conclusion. The validated method was applied in Iranian healthy subjects under fasting condition with a 50 mg riluzole tablet successfully.

Keywords: riluzole, amyotrophic lateral sclerosis, bioequivalence, LC-MS/MS, validation, pharmacokinetics

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Sevda Mohammadzadeh – writing the article. Tayebeh Ghari, Sima Sadrai – methodology and investigation. Tayebeh Ghari – software and statistical analysis. Faranak Salmannejad – review and editing. Mehdi Mohammadzadeh, Jalal Zaringhalam – supervisors.

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Compliance with the principles of ethics. The Iranian Registry of Clinical Trials approved the study protocol of the fasting condition with the IRCT ID of IRCT20200625047913N1.
No animals were used in this study. All the human experiments were conducted as per the guidelines of Helsinki declaration.

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INTRODUCTION

As an orphan and destructive disease of the nervous system, ALS can affect the brain and spinal cord nerve cells and ultimately lead to many problems, including muscle weakness and premature death [1, 2]. Unfortunately, most ALS patients do not survive more than 2 to 5 years due to the disease's progressive nature [3, 4]. Riluzole increases the average survival of patients by 3 to 6 months [5]. Usually, the dosage for riluzole tablets is 50 mg twice a day for the treatment of ALS. This report compares the pharmacokinetic parameters of riluzole 50 mg tablet manufactured by Hogar-Daroo versus Rilutek® 50 mg tablet manufactured by Sanofi Aventis Pharma to reveal the bioequivalence between the generic riluzole and the reference product.

To measure riluzole in plasma samples, we needed a valid method. When we reviewed the literature, we found that Narapusetti et al. developed an LC-MS/MS employing a protein precipitation method for sample preparation [6]. Vikram et al. used a solid phase extraction technique to develop an LC-MS/MS method for the analysis of riluzole, which required 500 µL of human plasma, and the LLOQ of the method was 5 ng/mL [7]. Finally, we found that Chando et al. developed a method for analyzing riluzole that used LC-MS/MS. The method required a minimum of 200 µL of human plasma, and the LLOQ of the method was 0.5 ng/mL [8]. Although the last method seemed suitable, it was not the answer to our work. Therefore, we developed another method for determining riluzole in human plasma using an LC- MS/MS device. Also, since riluzole has a high protein binding and causes a high matrix effect in LC-MS/MS analysis, we used a liquid-liquid extraction method to separate riluzole and internal standard from plasma, and the required volume of plasma for the process was low (100 microliters). The sensitivity of the method was 0.5 ng/ml as well.

MATERIALS AND METHODS

Chemicals and Reagents

Riluzole and carbamazepine USP reference standard were purchased commercially. HPLC-grade acetonitrile and methanol, analytical grade of formic acid, TBME (tertiary-butyl methyl ether) and NaOH were purchased from Merck KGaA (Darmstadt, Germany). All solutions were prepared with sterile deionized water (Millipore Company, USA). Human (K2 EDTA) plasma was obtained from Blood Transfusion Organization of Iran (Tehran, Iran).

The test product, Amyoger® 50 mg tablet (Batch No.: 005, Exp. Date: 10/2021), was manufactured by Hogar-Daroo. Co., Iran. The reference product, Rilutek® 50 mg tablet (Batch No.: 9L46E, Exp. Date: 02/2022), was manufactured by Sanofi Aventis Pharma Limited, UK.

Design of Study

The design of the study was as follows: open-label, single-dose, randomized, two sequences and two-period crossover. The generic riluzole 50 mg tablet of Hogar-Daroo Co., Iran, and the reference product, Rilutek® 50 mg tablet of Sanofi Aventis, UK, in normal, healthy males and females and adult subjects, were studied under fasting conditions[1]. The washout period was ten days between treatments[2]. This study was conducted at the bioequivalence study center, Hezareh Sevom laboratory (Tehran, Iran), in accordance with International Conference on Harmonization, E6 Good Clinical Practice (ICH, E6 GCP) guidelines[3], and local laws and regulations. The Iranian Registry of Clinical Trials approved the study protocol of the fasting condition with the IRCT ID of IRCT20200625047913N1.

The order of acquiring each subject's test and reference products during both study periods was determined according to a randomization plan rendered with SAS® software.

Subject Design

Twenty-four healthy Iranian adult volunteers for fasting conditions were randomized to participate in this study. The number of subjects was determined based on literature review and considering indicators such as power ≥80, significance level 5 %, and bioequivalence limits = 80.00–125.00 %. People aged 18 to 55 years with a body mass index (BMI) between 18–25 kg/m^2 were included in the study. Routine laboratory tests (blood and urine) were taken from all people. Also, people were subjected to physical examination and ECG tests to ensure their full health. All aspects (good and bad) of participating in the study were explained to the subjects and they read and signed an informed consent document before starting the study. All participants were insured during the survey[4, 5].

Blood Sampling and Storage

All participants took a single dose of riluzole orally with 200 mL of water. 3 ml blood samples were drawn before administering the drug to the volunteers and 1, 2, 3, 4, 4.5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 11, 12, 24, and 48 hours post-dose and collected in K2 EDTA collection

[1] Guidance on riluzole: US Food and Drug Administration. 2008. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Riluzole_tab_20599_RC11-04.pdf. Accessed: 01.12.2022.

[2] Rilutek® (riluzole) tablets: US Food and Drug Administration. 2008. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020599s011s012lbl.pdf. Accessed: 01.12.2022.

[3] Guidance for industry: E6 good clinical practice integrated addendum to ich e6(r1) Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). 2018 Available at: https://www.fda.gov/media/93884/download. Accessed: 01.12.2022.

[4] Guidance for industry: Food–effect bioavailability and fed bioequivalence studies Rockville, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2002. Available at: https://www.fda.gov/files/drugs/published/Food-Effect-Bioavailability-and-Fed-Bioequivalence-Studies.pdf. Accessed: 01.12.2022.

[5] Guidance for industry: Bioavailability and bioequivalence studies for orally administered drug products–general considerations Rockville, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2002. Available at: https://www.gmp-compliance.org/files/guidemgr/ucm154838.pdf. Accessed: 01.12.2022.

tubes. A centrifuge at 4000 rpm for 15 minutes at 4 °C was used to separate the plasma. The collected plasma was kept at –80 °C until use[1, 2].

Plasma Sample Preparation

Before starting the analysis, the frozen plasma was thawed and brought to room temperature. Then the samples were vortexed for ten seconds. Then, 100 microliters of human plasma containing carbamazepine (IS) with a concentration of 500 ng/ml were added to the samples and mixed. To these samples, 100 µL of 1 N NaOH was added and briefly vortexed, followed by adding 1.25 mL of TBME and, after capping, vortexed for 10 min. After centrifuging samples at 4,000 rpm and ambient temperature for about 15 min, The supernatant was transferred into tubes and dried under nitrogen at 40 °C. The dried residue was diluted with formic acid (0.1 %)/acetonitrile (60:40 v/v) and vortexed until wholly dissolved. The dissolved residue was transferred to an autosampler vial and injected (20 µl) into the chromatography system[3, 4].

Bioanalytical Method Development

The Agilent 1200 Series Gradient HPLC system fitted with a binary pump, an autosampler, and a solvent degasser is used in this study. As mobile phase, acetonitrile and methanol were tested in different ratios with buffers like ammonium acetate and ammonium formate and acid additives like formic acid and acetic acid in differing strengths. In addition, flow rates from 0.50 to 1 mL/min were also investigated to achieve good peak shape and shorter run time. An Agilent 6460 mass spectrometer fitted with an MS-MS detector was applied in positive ion mode to detect riluzole and the IS. The voltage of the ion spray was put at 5000 V. The compound parameters viz. the fragmentor potential and collision energy were 90/41 V and 81/27 V for riluzole and the IS, respectively.The multiple reaction monitoring modes (MRM) were utilized to quantify ion transitions m/z 235.0/165.9 and m/z 137.6/110 for riluzole and m/z 236.9/194 for the IS. Dwell time was selected at 200 ms. Processing of obtained data was done by Masshunter Quant software[5, 6] [9].

Preparation of Stock and Working Solutions of Riluzole and IS

Two methanolic standard stock solutions of riluzole were prepared separately (1 mg/mL). One of the stock solutions was used to prepare working standard solutions by diluting a mixture of methanol and water (50:50, V/V; diluent). The quality control (Q.C.) samples were prepared to determine accuracy and precision using the second stock solution. A methanolic stock solution of carbamazepine (1 mg/mL) was prepared and used for working standard preparation of carbamazepine (500 ng/mL)[7, 8] [9].

For the preparation of calibration samples, 900 µL of human plasma was mixed with the 100 µL working standard solution of the analyte to obtain riluzole concentration levels of 0.5, 2.5, 5, 10, 50, 150, 200, 300 ng/mL. Similarly, the quality control samples were prepared by using the standard drug at concentrations of 0.5 (lower limit of quantitation quality control, LLOQ

[1] Guidance for industry: Food–effect bioavailability and fed bioequivalence studies Rockville, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2002. Available at: https://www.fda.gov/files/drugs/published/Food-Effect-Bioavailability-and-Fed-Bioequivalence-Studies.pdf. Accessed: 01.12.2022.

[2] Guidance for industry: Bioavailability and bioequivalence studies for orally administered drug products–general considerations Rockville, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2002. Available at: https://www.gmp-compliance.org/files/guidemgr/ucm154838.pdf. Accessed: 01.12.2022.

[3] Guidance for industry: Food–effect bioavailability and fed bioequivalence studies Rockville, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2002. Available at: https://www.fda.gov/files/drugs/published/Food-Effect-Bioavailability-and-Fed-Bioequivalence-Studies.pdf. Accessed: 01.12.2022.

[4] Guidance for industry: Bioavailability and bioequivalence studies for orally administered drug products–general considerations Rockville, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2002. Available at: https://www.gmp-compliance.org/files/guidemgr/ucm154838.pdf. Accessed: 01.12.2022.

[5] Guidance for industry: Analytical procedures and methods validation for drugs and biologics Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). 2015. Available at: https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf. Accessed: 01.12.2022.

[6] Guidance for industry: Bioanalytical method validation Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). 2018. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed: 01.12.2022.

[7] Guidance for industry: Analytical procedures and methods validation for drugs and biologics Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). 2015. Available at: https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf. Accessed: 01.12.2022.

[8] Guidance for industry: Bioanalytical method validation Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). 2018. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed: 01.12.2022.

QC), 7.5 (low-quality control, LQC), 25 (medium-quality control, MQC1), 125 (MQC2) and 250 ng/mL (high-quality control, HQC[1, 2] [9].

Method Validation

The developed bioanalytical method was fully validated based on US-FDA guidelines on bioanalytical method validation. The calibration curve, linearity, accuracy, precision, matrix effect, extraction recovery, carry-over effect, and stability were assessed and then applied in the bioequivalence study of riluzole tablets[3, 4] [9–11].

Pharmacokinetics Data

Calculation of Pharmacokinetic Parameters

Pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, k_e, $t_{1/2}$) of human samples were calculated by pk pharmacokinetic software. The sampling duration was 3 to 5 times the drug's half-life. According to the FDA guidelines, the acceptable range for AUC ratio was considered higher than 80 %[5].

Statistical Analysis

Analysis of variance (ANOVA) and 90 % confidence intervals (90 % CIs) of the ratios test/reference was used to determine bioavailability parameters. The products were considered bioequivalent when the ratio of averages of log-transformed data fell within 80–125 % for AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, and T_{max}[6].

RESULT AND DISCUSSION

Method Development

In this study, the effect of various variables on the response of the LC-MS/MS technique was tested and investigated, and finally, an optimal method was developed for measuring riluzole in human plasma. A combination of 0.1 % formic acid and acetonitrile with different volume ratios was used as the mobile phase composition. Finally, when the ratio (60 : 40, V/V) was applied gradually at a flow rate of 0.9 mL/min, it showed the optimum separation, elution, and peak shape (Table 1).

Table 1. **Gradient HPLC method**

Time (min)	Mobile phase: A (Formic acid 0.1 %)	Mobile phase: B (Acetonitrile)	Flow rate (mL/min)
0	60	40	0.9
3.5	60	40	0.9
3.6	30	70	0.9
5.5	30	70	0.9
6	60	40	0.9

In this method, a C18 column (10 cm × 4.6 mm I.D., 5.0 µm particle) was used, and riluzole and IS were separated and eluted from each other in 2.054 min and 1.48 min, respectively (Figure 1). The volume of injection into the column was 20 microliters.

Positive ionization was generated on the analyte and IS utilizing an electrospray ionizer. Therefore, the precursor ion in the Q1 spectrum was the protonated form of the analyte and the IS, [M + H] + ion, which was used to obtain the Q3 product ion spectra. The most sensitive mass transfer ion spectra were from m/z 235.0 to 137.6 and from m/z 236.9 to 194.0 for riluzole and IS, respectively.

Method Validation

Linearity

Calibration curves were obtained by plotting the riluzole peak area / IS peak area against concentration. The analyte showed good linearity in the 0.5–300 ng/mL concentration range. The weighting factor (1/x and $1/x^2$) were fitted to obtain the most suitable concentration-signal relationship, and the $1/x^2$ model showed a better relationship. All curves showed an average correlation coefficient of 0.9981.

[1] Guidance for industry: Analytical procedures and methods validation for drugs and biologics Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). 2015. Available at: https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf. Accessed: 01.12.2022.

[2] Guidance for industry: Bioanalytical method validation Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). 2018. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed: 01.12.2022.

[3] Guidance for industry: Analytical procedures and methods validation for drugs and biologics Silver Spring, MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). 2015. Available at: https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf. Accessed: 01.12.2022.

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[5] Guidance for industry: Statistical approaches to establishing bioequivalence Rockville MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2001. Available at: https://www.fda.gov/media/70958/download. Accessed: 01.12.2022.

[6] Guidance for industry: Statistical approaches to establishing bioequivalence Rockville MD: US Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2001. Available at: https://www.fda.gov/media/70958/download. Accessed: 01.12.2022.

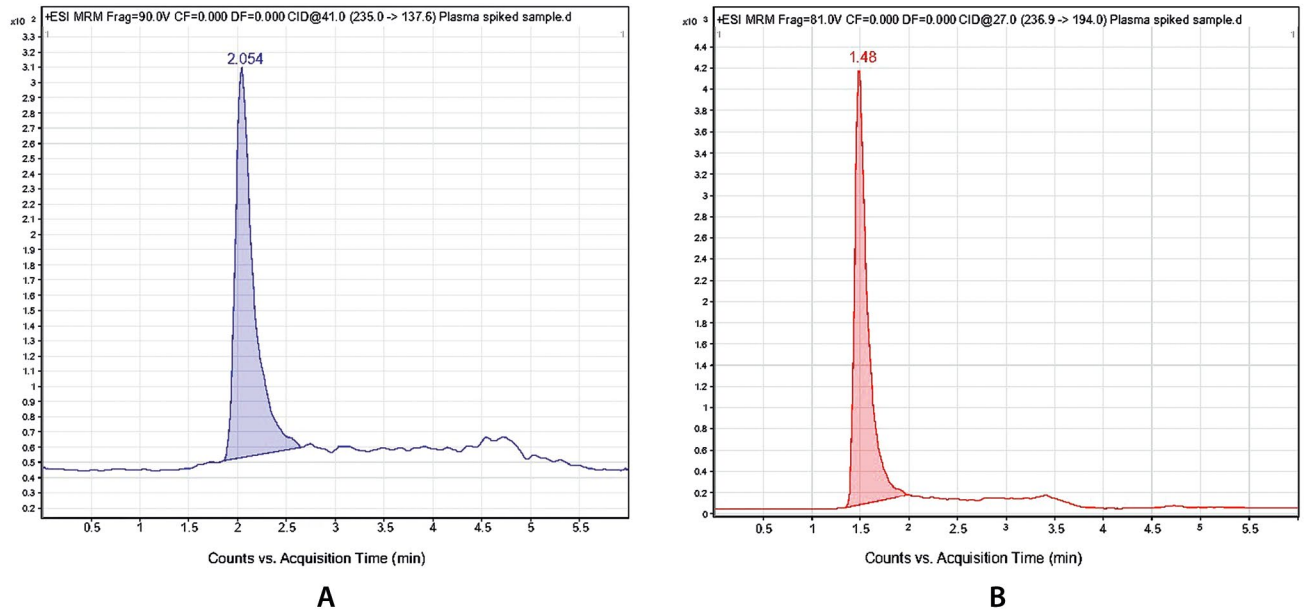

Figure 1. **Typical MRM chromatograms of riluzole (A) and the IS (B)**

Accuracy and Precision

Intra and inter-day accuracy and precision (%CV) of the current method were determined at four QC concentrations and LLOQ (Table 2). Intra-day accuracy was within 93.21 to 101.34 %, and inter-day accuracy was within 91.77 to 104.88 %. Intra-day precision ranged from 2.19 to 5.69 % and interday precision ranged from 1.67 to 5.31 %, all were in FDA acceptable range (±15 %). The current method has adequate accuracy, precision, and reproducibility for measuring riluzole in the plasma samples.

Table 2. **Intra and inter-day accuracy and precision (%CV) for riluzole**

Concentration (ng/mL)	Precision (% CV)	Accuracy (%)
Intra-day data (n = 6)		
0.5	5.69	96.31
7.5	4.23	98.75
25	2.19	101.34
125	3.56	100.62
250	2.98	93.21
Inter-day data (n = 30)		
0.5	5.31	98.89
7.5	3.21	102.55
25	1.67	91.77
125	4.56	100.31
250	3.66	104.88

Matrix Effect and Extraction Recovery

Since the plasma matrix may increase or decrease the ion signal, it should be considered to evaluate the ion signal strength [12, 13]. In this study, the rate of matrix effect was from 100.08 to 100.99, It shows that the plasma matrix had no pronounced effect on the ionization of the analyte (Table 3, Figure 2).

Table 3. **Matrix effect and recovery values for riluzole**

Concentration (ng/mL)	Matrix effect (%)	Recovery (%)
7.5	100.23	78
25	100.56	81
125	100.99	83
250	100.08	82

Since riluzole has high protein binding and binds to lipoproteins [14, 15], it is not easy to recover it from plasma. In the present method, we recovered up to 83 % (Table 3). Although this amount of recovery from plasma is considered relatively modest, given that the developed liquid-liquid extraction method is highly consistent (low CV values), we were able to use it to analyze samples confidently.

Carry over Effect

In order to check the effect of carryover on the current method, the highest concentration of analyte with is was injected into the device and after the injection of the blank sample, no effect of the previous injection was observed, which indicates the lack of influence of the analyzed sample and the internal standard on subsequent injections.

Stability

The stability results are depicted in Table 4. Riluzole was found stable in plasma at 25 ± 2 °C for 4 hours and three cycles of freeze-thaw. The stability of processed sample after 24 hours in the autosampler (25 ± 2 °C) was acceptable. The long-term stability of riluzole

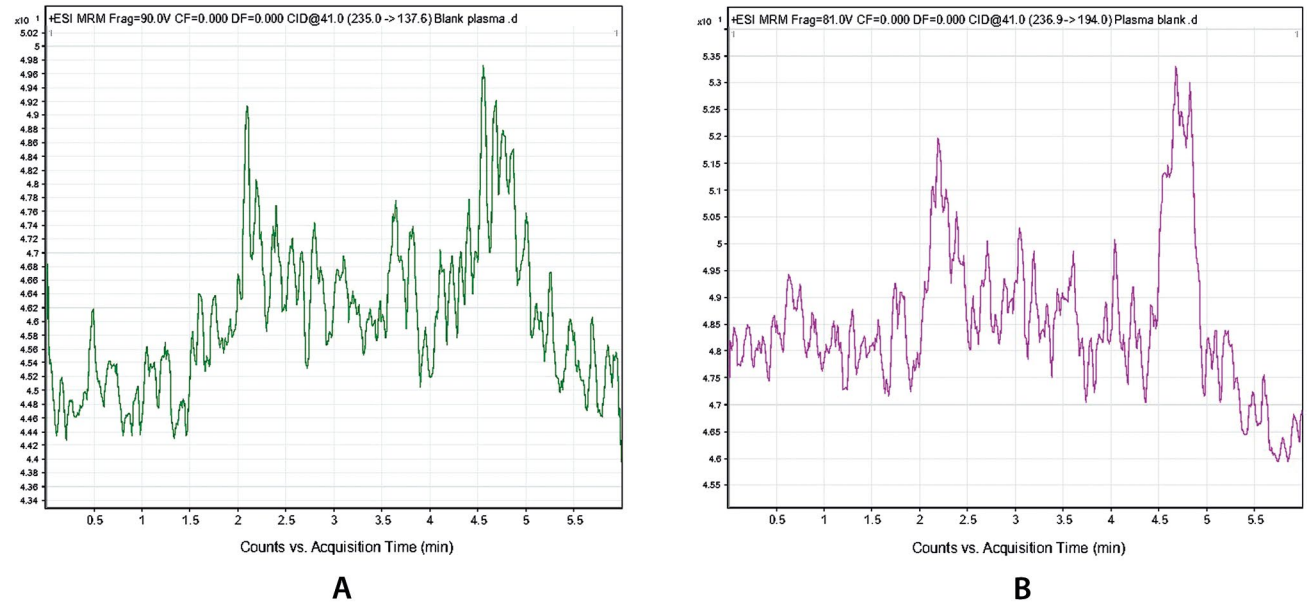

Figure 2. **Blank plasma spectra of riluzole (A) and carbamazepine (B)**

Table 4. **Stability testing of riluzole in plasma (n=6)**

Stability condition	Temperature	Concentration (ng/mL)					
		7.5		125		250	
		Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Bench-top (4 hr.)	Room temperature	94.12	5.93	93.81	3.71	98.34	2.35
Processed sample (24 hr.)	Room temperature	101.54	6.01	98.05	2.99	99.11	1.99
Freeze-Thaw (3 cycles)	–80 to room temperature	102.33	5.48	105.03	3.15	101.77	2.36
Long term (1 month)	–80	97.60	6.12	99.76	1.56	95.34	3.87

plasma samples showed its stability for one month at –80 °C. These results revealed that riluzole was stable for application in the routine analysis.

Calculation of Pharmacokinetic Parameters

The current method successfully quantified riluzole (50 mg) in blood samples of 24 healthy volunteers under fasting conditions. Figure 3 exhibits the mean plasma concentration versus time profile of riluzole. Table 5 shows mean pharmacokinetic parameters of riluzole from test and reference products. The test/reference ratio of the pharmacokinetic parameter is shown in Table 6.

Table 6. **The ratio of test/reference of pharmacokinetic parameters**

	Pharmacokinetic parameters (Ratio test/reference)					
	C_{max}	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	$t_{1/2}$	K_e
Mean	0.945	1.000	0.994	0.994	1.006	0.998
SD	0.038	0.000	0.044	0.041	0.065	0.069
CV (%)	4.012	0.000	4.451	4.142	6.469	6.911

Statistical analysis

The analysis of variance (ANOVA) and the 90 % confidence intervals (90 % CI) for the ratio of C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for the test and reference products are shown in Table 7.

Table 5. **Pharmacokinetic study results of riluzole (*n* = 24)**

Product		Pharmacokinetic parameters					
		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	$t_{1/2}$ (hr)	K_e (hr^{-1})
Test	Mean	157	0.9	692.0	766.4	13.8	0.051
	SD	19	0.3	33.0	25.6	1.5	0.007
	CV (%)	12	35.8	4.8	3.3	11.1	12.88
Reference	Mean	166	0.9	696.7	771.9	13.8	0.051
	SD	18	0.3	29.8	21.4	1.6	0.007
	CV (%)	11	35.8	4.3	2.8	11.6	12.88

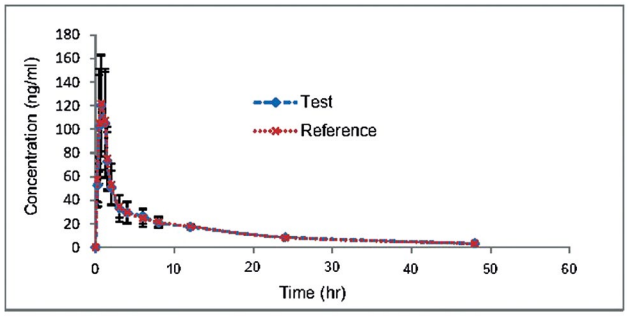

Figure 3. Linear plot of mean (±SD) plasma concentration of riluzole (one 50 mg tablet) versus time curves after oral administration of test product and reference product in healthy volunteers under fasting condition (*n* = 24)

Table 7. Analysis of variance (ANOVA) and the 90 % confidence intervals (90 % CI) for the ratio of Cmax, Tmax, AUC_{0-t} and $AUC_{0-\infty}$

Pharmacokinetic parameters	ANOVA (P-value)	90% confidence intervals for the ratio
C_{max}	0.09	0.93–0.96
T_{max}	1	1
AUC_{0-t}	0.61	0.98–1.01
$AUC_{0-\infty}$	0.42	0.98–1.01

For all pharmacokinetic parameters, the ratio of test/reference with 90 % confidence intervals (90 CIs) was within the acceptable range (80–125 %). P values for all parameters are >0.05 [16, 17]. Therefore, it can be supposed that test and brand riluzole tablets are biologically equivalent and can be used interchangeably.

CONCLUSION

This study developed a new analytical method for determining riluzole in plasma by LC-MS/MS. The amount of plasma used for liquid-liquid extraction was 100 microliters. Acceptable precision, accuracy, and sensitivity for estimating riluzole in human plasma samples were achieved by this method. The minimum detectable amount of riluzole with this method was 0.5 ng/ml, and this method was successfully used to study the bioequivalence of 50 mg riluzole tablets in Iranian volunteers under fasting conditions.

REFERENCES

1. Qiu Y., Chen Y., Zhang G., Yu L., Mantri R. V., editors. Developing solid oral dosage forms. 2 edition. Boston: Academic Press; 2017. P. 1127–1160.
2. Doble A. The pharmacology and mechanism of action of riluzole. *Neurology*. 1996;47(6–4):S233–S241. DOI: 10.1212/wnl.47.6_suppl_4.233s.
3. Longinetti E., Fang F. Epidemiology of amyotrophic lateral sclerosis: An update of recent literature. *Curr Opin Neurol*. 2019;32(5):771–776. DOI: 10.1097/wco.0000000000000730.
4. Masrori P., Van Damme P. Amyotrophic lateral sclerosis: A clinical review. *Eur J Neurol*. 2020;27(10):1918–1929. DOI: 10.1111/ene.14393.
5. Oskarsson B., Gendron T. F., Staff N. P. Amyotrophic lateral sclerosis: An update for 2018. *Mayo Clin Proc*. 2018;93(11):1617–1628. DOI: 10.1016/j.mayocp.2018.04.007.
6. Narapusetti A., Bethanabhatla S. S., Sockalingam A., Pilli N. R., Repakab N., Allaf T. Bioanalysis of riluzole in human plasma by a sensitive lc-ms/ms method and its application to a pharmacokinetic study in south indian subjects. *Analytical Methods*. 2014;6(13):4823–4830. DOI: 10.1039/C4AY00583J.
7. Vikram P., Shanmugasundaram P. Determination of riluzole in human plasma by ultra performance liquid chromatography-tandem mass spectrometry (uplc-ms/ms) and its application to a pharmacokinetic study. *Research Journal of Pharmacy and Technology*. 2017;10(1):193–199. DOI: 10.5958/0974-360X.2017.00042.7.
8. Chandu B. R., Nama S., Kanala K., Challa B. R., Shaik R. P., Khagga M. Quantitative estimation of riluzole in human plasma by LC-ESI-MS/MS and its application to a bioequivalence study. *Anal Bioanal Chem*. 2010;398(3):1367–1374. DOI: 10.1007/s00216-010-4034-8.
9. Zimmer D. New us fda draft guidance on bioanalytical method validation versus current fda and ema guidelines: Chromatographic methods and isr. *Bioanalysis*. 2014;6(1):13–19. DOI: 10.4155/bio.13.298.
10. Karpova P. A., Komarov T. N., Archakova O. A., Shchelgacheva D. S., Suvorova A. V., Bagaeva N. S., Karnakova P. K., Shohin I. E. Development and validation of a method for determining deferasirox in human blood plasma by HPLC-UV. *Drug development and registration*. 2022;11(2):187–196. DOI: 10.33380/2305-2066-2022-11-2-187-196.
11. Matuszewski B. K., Constanzer M. L., Chavez-Eng C. M. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem*. 2003;75(13):3019–3030. DOI: 10.1021/ac020361s.
12. Caban M., Migowska N., Stepnowski P., Kwiatkowski M., Kumirska J. Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of β-blockers and β-agonists in environmental samples. *Journal of Chromatography A*. 2012;1258:117–127. DOI: 10.1016/j.chroma.2012.08.029.
13. Chow D. S., Teng Y., Toups E. G., Aarabi B., Harrop J. S., Shaffrey C. I., Johnson M. M., Boakye M., Frankowski R. F., Fehlings M. G., Grossman R. G. Pharmacology of riluzole in acute spinal cord injury. *Journal of Neurosurgery: Spine*. 2012;17(1):129–140. DOI: 10.3171/2012.5.Aospine12112.
14. Nagoshi N., Nakashima H., Fehlings M. G. Riluzole as a neuroprotective drug for spinal cord injury: From bench to bedside. *Molecules*. 2015;20(5):7775–7789. DOI: 10.3390/molecules20057775.
15. Wagner M. L., Landis B. E. Riluzole: A new agent for amyotrophic lateral sclerosis. *Ann Pharmacother*. 1997;31(6):738–744. DOI: 10.1177/106002809703100614.
16. Chow S. C. Bioavailability and bioequivalence in drug development. *Wiley Interdisciplinary Reviews: Computational Statistics*. 2014;6(4):304–312. DOI: 10.1002/wics.1310.
17. Mathew T. Bioequivalence studies in drug development: Methods and applications by dieter hauschke, volker steinijans, iris pigeot. *International Statistical Review*. 2007;75(2):272–273. DOI: 10.1111/j.1751-5823.2007.00015_24.x.