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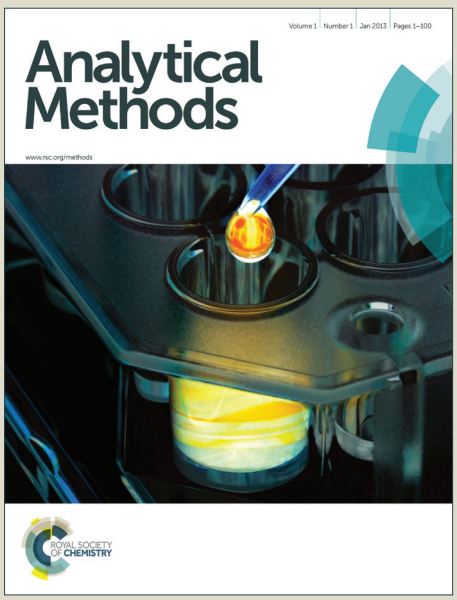

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Bioanalysis of riluzole in human plasma by a sensitive LC–MS/MS method and its application to pharmacokinetic study in South Indian subjects

Anjaneyulu Narapusetti [a, b *], Syama Sundar Bethanabhatla [c], Anbazhagan Sockalingam [d], Nageswara Rao Pilli [e], Nagakishore Repaka [b], Tejasri Alla [f]

[a] Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Kakinada, Kakinada–533 003, India

[b] Department of Pharmaceutical Analysis and Pharmacology, Geethanjali College of pharmacy, Cheeryal–501 301, India.

[c] Department of Chemistry, Yogi Vemana University, Kadapa–516 003, India

[d] Department of Pharmaceutical Chemistry, Surya School of Pharmacy, NH-45, GST Road, , Vikravandi, Villpuram–605 652, India

[e] Center for Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad–500 085, India

[f] Department of Pharmaceutical Chemistry, Holly Mary Institute of Technology & Science College of Pharmacy, Bogaram–501 301, India

***Correspondence to**
Anjaneyulu Narapusetti, Department of Pharmaceutical Analysis, Geethanjali College of pharmacy, Cheeryal–501 301, India.

E–mail: anjipharmacy@gmail.com

Tel.: +91–9959967431

Running title: LC–MS/MS determination of riluzole in human plasma.

Abstract

In this paper, the authors proposed a simple, rapid and highly sensitive liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay method for the determination of riluzole in human plasma. Carbamazepine was used as an internal standard (IS). The method employed only 100 µL of human plasma for sample processing by simple protein precipitation technique. The processed samples were chromatographed on a C_{18} column by using a mixture of 0.1% formic acid – acetonitrile (30:70, v/v) as the mobile phase at a flow rate of 0.9 mL/min. The calibration curve obtained was linear over the concentration range of 0.10–500 ng/mL with $r^2 \geq 0.99$. Method validation was performed as per FDA guidelines and the results met the acceptance criteria. The multiple reaction–monitoring mode (MRM) was used for quantification of ion transitions at *m/z* 235.0/165.9 and 237.2/194.1 for the analyte and the IS, respectively. A run time of 2.0 min for each sample made it possible to analyze more than 400 plasma samples per day, thus increasing the productivity. The validated method was successfully applied to a clinical pharmacokinetic study in South Indian male Subjects under fasting condition with 50 mg riluzole tablet.

Keywords:

Riluzole, Human plasma, Protein precipitation (PP), LC–MS/MS, Pharmacokinetics

Introduction

Riluzole (Fig 1.), a orally available antiglutamatergic agent used in the treatment of numerous diseases including amyotrophic lateral sclerosis (ALS)/ motor neuron disease (MND) [1] Parkinson's disease (PD) [2], Huntington's disease (HD) [3], mood and anxiety disorders and multiple sclerosis (MS) [4]. The drug is extensively metabolized by cytochrome P450 1A2 (by hydroxylation and glucuronidation) in the liver [5]. After oral administration, the absolute bioavailability of riluzole is reported to be 60%. The drug has high protein binding nature (about 96%) to plasma proteins [6].

As per the literature, few high performance liquid chromatographic (HPLC) methods have been reported for the determination of riluzole in a variety of biological samples like rat brain [7], rat plasma [8, 9] and in human plasma [10, 11]. Most of these reported methods [7–9] were suitable for pre–clinical application in animal models. HPLC still remains a method of choice, as it is able to separate quite complicated mixtures of low– and high molecular weight compounds, as well as different polarities and acid–base properties in various matrices. Unfortunately, conventional HPLC methods must sacrifice time, resolution or sensitivity. Therefore, it is necessary to develop fast or ultra–fast methods such as LC–MS/MS without any loss of separation efficiency.

Similarly, only one liquid chromatography tandem mass spectrometric (LC–MS/MS) method has been reported for the determination of riluzole in human plasma. Chandu *et al.,* (2010) **[12]** reported a LC–MS/MS method with plasma concentration range of 0.5–500 ng/mL. This method employs liquid–liquid (L–L) extract, evaporation, drying and reconstitution for sample preparation; however, not sensitive enough for the

determination of riluzole concentrations for pharmacokinetic/bioequivalence studies because of its higher LLOQ. Post–dosing quantitation of any drug during terminal phase is very critical to obtain key pharmacokinetic parameters. Simple and efficient method with high sensitivity is required to quantify the drug concentrations at terminal phase. Currently, the aim of bioanalytical scientists is to develop reliable, rapid and efficient procedures for performing qualitative and quantitative analysis.

The present paper describes a simple, rapid and highly sensitive liquid chromatography with electrospray ionization–tandem mass spectrometric method, which employs one step protein precipitation technique (PP) for sample preparation for the quantitation of riluzole in human plasma with a chromatographic run time 2.0 min. In the present investigation we have achieved a higher sensitivity (5 fold) using a low plasma volume (100 µL) compared with earlier reports [12]. The method ensured the estimation of riluzole in real time samples collected from healthy male subjects up to 72 h of post dosing with desired accuracy and precision to support a pharmacokinetic study in healthy volunteers. Furthermore, for the first time, assay reproducibility is demonstrated through incurred sample reanalysis (ISR).

Experimental

Standards and chemicals

The reference sample of riluzole (99.80%) was obtained from Clearsynth Labs Limited (Mumbai, India), while carbamazepine (99.8%) was from Neucon Pharma Pvt. Ltd., (Goa, India). Water used for the LC–MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). HPLC grade methanol and acetonitrile were purchased from J.T. Baker (Phillipsburg, USA), while analytical grade formic acid was from Merck Ltd (Mumbai, India). The control K_2 human plasma sample was procured from Deccan's Pathological Lab's (Hyderabad, India).

LC–MS/MS instrument and conditions

An HPLC system (Shimadzu, Kyoto, Japan) equipped with a Ace 5 C_{18} column (50 mm × 4.6 mm, 5 µm) (Make: Ace HPLC columns), a binary LC–20AD prominence pump, an auto sampler (SIL–HTc) and a solvent degasser (DGU–$20A_3$) was used for the study. Aliquot of 20 µL of the processed samples were injected into the column, which was kept at ambient (20±5°C) temperature. An isocratic mobile phase composed of a mixture of 0.1% formic acid and acetonitrile (30:70, v/v) was used to separate the analyte from the endogenous components and pumped at a flow rate of 0.90 mL/min into the electrospray ionization chamber of the mass spectrometer. Quantification was achieved with MS–MS detection in positive ion mode for the analyte and the IS using an AB Sciex API–4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray ™ interface at 500 °C. The ion spray voltage was set at 5000 V. The source parameters viz. the nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 40,

35, 35, and 8 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were 90, 41, 10, 11 V for riluzole and 81, 27, 10, 11 V for the IS. Detection of the ions was carried out in the multiple–reaction monitoring mode (MRM), by monitoring the transition pairs of *m/z* 235.0 precursor ion to the *m/z* 165.9 for riluzole and *m/z* 237.2 precursor ion to the *m/z* 194.1 product ion for the IS. Quadrupoles (Q1 and Q3) were set on unit resolution. Dwell time was set at 200 ms. The analysis data obtained were processed by Analyst software™ (version 1.6.1).

Preparation of stock and working solutions

Two standard stock solutions of riluzole were prepared separately in HPLC grade methanol (1 mg/mL). Their concentrations were corrected according to the actual amount weighted accounting for its potency. Working standard solutions necessary for plotting the calibration curve (CC) samples were prepared by appropriate dilution of the one of the above stock solution of the riluzole using a mixture of methanol and water (50:50, v/v; diluent). Quality control (QC) samples for determination of accuracy and precision were prepared by appropriate dilution of the second standard stock solution prepared above using the same diluent. The concentrations of the QC samples are selected from the five different levels of the calibration curve range.

A 1 mg/mL of carbamazepine stock solution was prepared by dissolving the compound in HPLC grade methanol. The working concentration of carbamazepine (500 ng/mL) was prepared from the above stock solution using the diluent.

Preparation of calibration curve standards and quality control samples in human plasma

Six lots of K_2 EDTA human plasma were screened and used to prepare calibration curve standards, quality control samples and dilution integrity (DIQC) samples. After bulk spiking, aliquots of 200 µL for CCs and 200 µL for QCs of spiked plasma samples were pipetted out into a prelabelled micro centrifuge tubes (2 mL) and then all the bulk spiked samples were stored in deep freezer at –70±10 °C. Additionally, twelve sets of LQC and HQC were stored at –20±5°C to check stability at –20°C.

Calibration samples were prepared by spiking 950 µL of control K_2 EDTA human plasma with the 50 µL working standard solution of the analyte as a bulk, to obtain riluzole concentration levels of 0.10, 0.20, 1.02, 10.2, 25.5, 75.1, 150, 300, 400 and 500 ng/mL as a single batch at each concentration. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drug, at concentrations of 0.105 (lower limit of quantitation quality control, LLOQ QC), 0.30 (low quality control, LQC), 60.2 (medium quality control, MQC1), 251 (MQC2) and 448 ng/mL (high quality control, HQC) as a single batch at each concentration.

Sample preparation protocol

All frozen subject samples, calibration standards and quality control samples were thawed and allowed to equilibrate at room temperature prior to analysis. The samples were vortexed to mix for 10 s prior to spiking. A 100 µL aliquot of human plasma sample was mixed with 20 µL of the internal standard working solution (500 ng/mL of carbamazepine). To this, 50 µL of the 5% formic acid buffer and 1.0 mL of acetonitrile

were added. After vortex–mixing for 30 s and centrifugation at 4000 rpm for 15 min, the supernatant was transferred to another clean test tube and evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was reconstituted with 250–µL of the mobile phase and 20 µL were injected into LC–MS/MS system.

Method validation procedures

The validation of the above method was carried out as per US FDA guidelines [13]. System suitability test was performed by injecting six repeated injections of aqueous mixture of the analyte and the IS. Carryover experiment was performed to verify any carryover of analyte and the IS, which may reflect in subsequent runs. The carryover test samples were injected in the following sequence i.e. blank plasma sample → six samples of LLOQ → blank plasma sample → ULOQ sample → blank plasma samples to check the carryover effect. The selectivity of the method was assessed in six different sources of plasma, of which, four were normal K_2 EDTA plasma and one each of lipemic and helolyzed plasma. Sensitivity of the method was assessed by analyzing six sets of spiked plasma samples at lowest level of the calibration curve concentrations (LLOQ). Matrix effect, expressed as IS normalized matrix factor (MF) was assessed by comparing the mean area response of post–extraction spiked samples with mean area of aqueous samples (neat samples) prepared in mobile phase solutions at LQC and HQC levels. The overall precision of the matrix factor was expressed as coefficient of variation (CV).

$$\text{Matrix: } = \frac{\text{Peak response area ratio in presence of matrix ions}}{\text{Mean peak response area ratio in absence of matrix ions}}$$

Matrix effect was also evaluated with six different lots of K_2 EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total).

The linearity of the method was determined by analysis of five standard calibration curves (CC) containing ten non–zero concentrations. In addition, each curve contains one blank plasma sample and one blank plasma sample with internal standard (zero standard). Each CC was analyzed individually by least square weighted ($1/x^2$) linear regression. Intra–day accuracy and precision was determined using six replicates of LLOQ QC, LQC, MQC–1, MQC–2 and HQC samples were analyzed along with a calibration curve in a single day. Inter–day accuracy and precision were assessed by analyzing three batches of samples on two consecutive days. The precision (% CV) at each concentration level from the nominal concentration should not be greater than 15%, except for LLOQ QC where it should be 20%. The accuracy (%) must be within ±15% of their nominal value at each QC level except LLOQ QC where it must be within ±20%.

Recovery for the analyte and the IS was calculated by comparing the mean detector response of six sets of pre–extraction spiked samples (spiked before extraction) to that of six sets of neat samples (aqueous) at each concentration level. Recovery of riluzole was determined at a concentration of 0.30 (LQC), 251 (MQC2) and 448 (HQC) ng/mL, whereas for the IS was determined at concentration of 500 ng/mL.

Stock solution stability of the analyte and the IS was tested at room temperature for 12 h and at 2–8 °C in refrigerator for 28 days. The stock solution stability was performed by comparing the area response stability samples with the response of the

sample prepared from fresh stock solution. The solutions were considered stable if the deviation within ±10% from the nominal value. Bench top stability at room temperature (20 h), processed samples stability (autosampler stability for 72 h, wet extract stability for 68 h and reinjection stability for 45 h), freeze–thaw stability (4 cycles) were performed at LQC and HQC levels using six replicates at each level. Similarly, the long term stability (at –70±10°C for 90 days) and short term stability (at –20±5°C for 8 days) of spiked plasma samples was also studied at both the QC levels. The stability samples were processed and quantified against freshly spiked calibration curve standards along with freshly spiked QC samples. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (≤15% RSD).

The method ruggedness was also established by analyzing one precision and accuracy batch on different column of the same make (different batch no.) using different set of reagents processed by different analyst. Dilution reliability was performed to extend the upper concentration limit with acceptable precision and accuracy. Six replicates each at a concentration of about 1.66 times of the uppermost calibration standard were diluted two– and four–fold with screened blank plasma. The diluted samples were processed and analyzed with un–diluted calibration curve samples.

Pharmacokinetic study protocol and incurred samples reanalysis

The proposed method was applied to determine riluzole plasma concentration for a pharmacokinetic study conducted in 6 healthy Indian subjects. South Indian healthy male subjects with an age group of 20–40 years and body–mass index (BMI) of ≥18.5

kg/m^2 and ≤24.9 kg/m^2, with body weight not less than 50 kg were selected for the study. All the volunteers provided with written informed consent and were fasted for 12 h before the drug formulation administration. The subjects were orally administered a single dose of riluzole hydrochloride (50 mg tablet) with 200 mL of water. Blood samples were withdrawn at pre–dose and 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48 and 72 h and collected in K_2 EDTA vacutainer (5 mL) collection tubes (BD, Franklin, NJ, USA). The tubes were centrifuged at 3200 rpm for 10 min and the plasma was collected. The collected plasma samples were stored at –70 ± 10 °C till the usage. Plasma samples were spiked with the IS and processed as per the extraction procedure described earlier. The main pharmacokinetic parameters of riluzole were calculated by non–compartmental model using WinNonlin Version 5.2. An ISR was also performed by selecting the 12 subject samples (2 samples from each subject) near C_{max} and the elimination phase in the pharmacokinetic profile of the drug. The ISR values were compared with the initial values. The percent change in the value should not be more than ±20% [14, 15].

Results and discussion

Method development

The current method was developed using electrospray ionization source in the positive ionization mode. During method development the analyte and the IS were tuned in positive and negative ionization modes using tuning solution (100 ng/mL), but the response obtained in positive mode much higher than the negative mode. Protonated form of analyte and the IS, $[M+H]^+$ ion was the precursor ion in the Q_1 spectrum and was

used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from *m/z* 235.0 to 165.9 for riluzole and from *m/z* 237.2 to 194.1 for the IS. The most intense and consistent product ion Q3 MS spectra of analyte and the IS was obtained by optimizing the collision energy and collision cell exit potential. The source parameters like nebulizer gas (GS1), auxiliary gas (GS2), collision gas, temperature and ion spray voltage were optimized to obtained adequate and reproducible response for the analyte. The dwell time for each transition was set at 200 ms. The product ion mass spectra of riluzole was presented in the Fig 2. As earlier publications have discussed the details of fragmentation patterns of carbamazepine [16] we are not presenting the data pertaining to this. The LC–MRM technique was chosen for the assay development due its inherent selectivity and sensitivity.

The method development includes mobile phase selection, column type, flow rate, and injection volume. Acetonitrile and methanol were tried in different ratio with buffers like ammonium acetate, ammonium formate as well as acid additives like formic acid and acetic acid in varying strength. It was observed that 0.1% formic acid and acetonitrile (30:70, v/v) as the mobile phase was most appropriate to give best sensitivity, efficiency and peak shape. Addition of small amount of formic acid helped to improve the peak shape and spectral response. 30% aqueous part was adequate to retain the riluzole and the IS. The use of a short chromatography column Ace 5 C_{18} (50 mm × 4.6 mm, 5 µm) helped in the separation and elution of all three compounds in a very short time. The total chromatographic run time was 2.0 min for each run. In addition, the effect of flow rate was also studied from 0.50 to 1.2 mL/min, which was

also responsible for acceptable chromatographic peak shape and short run time and finally flow rate was set at 0.90 mL/min. The retention time of riluzole and the IS obtained with the above optimized chromatographic conditions were low enough (0.95 and 0.85 min) allowing short run time of 2.0 min.

Biological samples are complex and contain many endogenous components. To develop a sensitive (ng or pg level) analytical method in biological samples one should have a proper extraction technique which can produce good recovery with minimal or no matrix effect. The earlier authors [12] have employed LLE to extract riluzole from human plasma samples. Therefore, protein precipitation (PP) was carried out using ethanol, methanol, acetonitrile and the mobile phase as precipitating agents under acidic and basic conditions. Also, riluzole had more protein binding nature and were precipitated easily with the single protein precipitant. But, although methanol and ethanol gave good results, the recovery was not consistent at LQC level due to ion suppression. Also, chromatography was not acceptable at lowest concentration level. Finally, promising results were obtained with acetonitrile, which can produce a clean chromatogram for a blank sample and yields the maximum recovery for the analyte from the plasma. Addition 5% formic acid to the plasma samples as an extraction additive helped achieving reproducible and quantitative recoveries for the analyte and the IS. Initially, supernatant was injected directly in to the LC system, but the peak shape of analyte was unacceptable at lower concentration levels and also response was insufficient to quantify the analyte. Hence, the supernatant was evaporated and the residue was reconstituted with the mobile phase. The overall mean recoveries of

analyte and the IS were good and reproducible. In addition, the method validation results and pharmacokinetic study support this extraction methodology and hence it was used in the present study.

An ideal internal standard should mimic the analyte during ionization, chromatography and extraction. Stable isotope–labeled drugs or deuterated compounds are preferred internal standards for LC–MS/MS analysis. But these compounds are expensive and/or not available to serve as IS. So, at the initial stages of this work, many compounds were investigated in order to find suitable IS, finally carbamazepine was selected, based on the chromatographic elution, ionization and extraction efficiency. Moreover, the validation results encouraged its selection as an internal standard.

System suitability and carryover test

The precision (% CV) for system suitability test was found to be in the range of 0.00–0.55% for the retention time of riluzole and 0.00–0.48% for the IS and 0.54–1.82% for the area response of riluzole and the IS.

Carryover was evaluated to ensure that it does not affect the accuracy and precision of the proposed method. No significant carryover was observed in blank sample after injection highest concentration of analyte along with the working concentration of the IS (ULQ; upper limit of quantitation) which indicates no carryover of the analyte and the IS in subsequent samples (data not publicized).

Selectivity and chromatography

The method selectivity was evaluated by analyzing blank human plasma extract (Fig. 2A) and an extract spiked only with the IS (Fig. 2B). As shown in Fig. 2A, no significant direct interference in the blank plasma traces was observed from endogenous components at the retention time of the analyte and the IS. Similarly, Fig. 2B shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 2C depicts a representative ion–chromatogram for the LLOQ sample (0.10 ng/mL). Likewise, no interference was observed from commonly used drugs such as paracetmol, nicotine, pantoprazole, ibuprofen, caffeine, diphenhydramine, dicyclomine and pseudoephedrine (data not presented). Fig. 3 depicts a representative chromatograms resulting from the analysis of subject blank plasma sample and 5 h subject plasma sample after the single oral dose of a riluzole 50 mg tablet.

Sensitivity

LLOQ is the lowest limit of reliable quantification for the analyte and was set at 0.10 ng/mL. At this concentration the signal–to–noise ratio (S/N) was measure and found to be ≥10. The precision and accuracy at LLOQ concentration were found to be 5.83% and 106%, respectively.

Matrix effect

Matrix effect assessment was done with the aim to check the effect of different lots of plasma on the back calculated value of QC's nominal concentration. As shown in Table 1, no significant matrix effect was observed in the six batches of human plasma lots screened for the analyte at both the concentration levels (LQC and HQC) and the results

found were well within the acceptable limits. Also, the extraction method was rugged enough and gave accurate and consistent results when applied to real subject samples for a pharmacokinetic study.

Linearity, precision and accuracy

The analyte showed good linearity in the concentration rage of 0.10–500 ng/mL. Both the regression models ($1/x$ and $1/x^2$) were compared and best fit for the concentration–detector response relationship was obtained with a weighting factor of $1/x^2$. The mean correlation coefficient values were in the range of 0.9946–0.9978 for all the analytical runs generated during entire course of validation.

Table 2 summarizes the intra–day and inter–day precision and accuracy results of riluzole for five precision and accuracy batches in plasma at five QC concentration levels. The precision (% CV) and accuracy values of riluzole for intra– and inter–day ranged from 2.14–6.44% and 92.2–106%, and 2.54–6.05% and 91.8–106%, respectively. The results revealed good precision and accuracy.

Extraction efficiency and dilution integrity

With the proposed protein precipitation method, the mean overall recovery obtained for riluzole was 95.7±3.63% with the precision range of 2.18–5.24% and for the IS was 86.3% with the precision range of 3.23–4.17%. Good and reproducible recoveries were obtained for the analyte and the IS. Hence, the assay has been proved to be robust in high throughput bioanalysis.

The upper concentration limit of riluzole can be extended to 828 ng/mL by using half (1:2) or quarter (1:4) dilution with screened human blank plasma. The precision

(%CV) for dilution integrity of half and quarter dilution was found to be 4.03% and 1.72%, while the accuracy results were found to be 94.6% and 93.7%, respectively.

Ruggedness and long run evaluation

Ruggedness of the present method was established with one precision and accuracy batch. Precision and accuracy batch–V was processed by the different analyst and analyzed on the different instrument of the same make using different set of reagents and different column (different batch no.). The precision (%CV) and accuracy values for ruggedness were ranged from 0.83–6.27% and 89.6–105%, respectively.

Run size evaluation was carried out to assess the integrity of the samples analyzed in a long run during study sample analysis. 40 sets of each of LQC, MQC1, MQC2 and HQC samples were processed and analyzed for run size evaluation along with freshly spiked calibration curve standards and quality control samples. 160 QC's out of 160 QC's of run size evaluation and 24 QC's out of 24 QC's of freshly prepared were within 15% of their respective nominal (theoretical) values. The %CV (precision) and accuracy results for run size evaluation QC's were ranged from 0.93–2.39% and 91.7–98.4%, respectively. Similarly, the %CV (precision) and accuracy results for freshly prepared QC's were ranged from 0.89–1.66% and 98.3–106%, respectively.

Stability studies

Analyte stability at various conditions was evaluated. In the different stability experiments carried out viz. bench top stability (20 h), autosampler stability (72 h), wet extract stability (68 h), repeated freeze–thaw cycles (4 cycles), reinjection stability (45 h) and long term stability at –70±10°C for 90 days the mean % nominal values of the

analyte were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels (Table 3). Therefore, the results were found to be within the acceptable limits during the entire validation.

Stock solutions of riluzole and the IS were found to be stable for 28 days in refrigerator at 2–8 °C and 12 h at room temperature (20±5°C). The percentage stability (with the precision range) of riluzole and the IS at 2–8 °C was 99.2% (0.70–0.79%) and 98.7% (0.36–0.89%), respectively. Similarly, the percentage stability (with the precision range) of riluzole and the IS at room temperature was 96.6% (2.07–3.36%) and 97.0% (1.78–2.84%), respectively.

Pharmacokinetic study and incurred samples reanalysis

The present method was lucratively used to determine riluzole plasma concentrations for a pharmacokinetic study in healthy South Indian adult male subjects (*n*=6). Fig. 4 depicts the mean plasma concentration *vs* time profile of riluzole (presented up to 12 h in order to depict the plot with clarity) after oral administration of a 50 mg dose of riluzole tablet under fasting condition. The maximum concentration (C_{max}) in plasma (170±26.2 ng/mL) for riluzole was attained at 1.12±0.40 h (t_{max}). The area under the plasma concentration–time curve from time zero to last measurable time point (AUC_{0-t}) and area under the plasma concentration time curve from time zero to infinity time point (AUC_{0-inf}) for riluzole were 777±196 and 783±196 ng*h/mL, respectively. The terminal half–life (t½) was found to be 11.9±3.18 h. These values were in close proximity when compared with earlier reported values [12].

The authenticity of the proposed method was established by reanalysis of incurred samples (ISR). For ISR two plasma samples from each subject were selected and re–analyzed in a single bioanalytical run. The differences in concentrations between the ISR and the initial values for all the tested samples were less than 20% (Table 4), indicating good reproducibility of the present method.

Conclusions

The proposed LC–MS/MS assay method is simple, rapid, specific and highly sensitive for the quantification of riluzole in human plasma and is fully validated according to commonly acceptable FDA guidelines. This method is highly sensitive and employs only 100 µL plasma volumes for sample processing. The simple PP with acetonitrile method gave consistent and reproducible recoveries for the analyte and the IS from plasma. Moreover, the total analysis time (extraction and chromatography) is the shortest compared to all these methods. Thus, the advantage of this method is that a relatively large number of samples can be analyzed in short time, thus increasing the output. The method provided good linearity. The stability of the analyte in plasma and in aqueous samples under different conditions has been extensively studied and is meeting acceptance criteria. The method was found to be reliable and reproducible to support pharmacokinetic study in humans. From the results of all the validation parameters, we can conclude that the developed method can be useful for bioavailability and bioequivalence (BA/BE) studies and routine therapeutic drug monitoring with the desired precision and accuracy.

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Table 1

Matrix effect assessment results of riluzole in human plasma (*n* = 3)

Plasma lot	LQC (0.30 ng/mL)			HQC (448 ng/mL)		
	Concentration found (mean ± SD; ng/mL)	% Accuracy	IS normalized MF	Concentration found (mean ± SD; ng/mL)	% Accuracy	IS normalized MF
Lot 1	0.32 ± 0.01	107	1.01	411 ± 7.49	91.6	1.10
Lot 2	0.31 ± 0.02	104	1.01	426 ± 13.5	95.1	1.10
Lot 3	0.32 ± 0.01	106	1.03	415 ± 12.0	92.7	1.10
Lot 4	0.32 ± 0.02	108	1.02	418 ± 5.74	93.3	1.09
Lot 5	0.31 ± 0.02	104	1.03	421 ± 6.86	93.9	1.09
Lot 6	0.32 ± 0.02	106	1.00	426 ± 13.7	95.1	1.08

Table 2

Precision and accuracy data for riluzole

Quality control	Run	Concentration found (mean ± SD; ng/mL)	Precision (%)	Accuracy (%)
Intra–day variations (Six replicates at each concentration)				
LLOQ	1	0.108 ± 0.006	5.43	103
	2	0.109 ± 0.004	3.86	104
	3	0.110 ± 0.007	6.40	105
	4	0.118 ± 0.008	6.72	112
	5	0.110 ± 0.005	4.95	105
LQC	1	0.32 ± 0.02	6.86	106
	2	0.30 ± 0.01	2.99	98.1
	3	0.32 ± 0.02	4.81	106
	4	0.32 ± 0.02	6.47	105
	5	0.31 ± 0.02	6.27	104
MQC1	1	64.4 ± 2.39	3.72	107
	2	63.5 ± 1.07	1.69	106
	3	65.0 ± 1.41	2.17	108
	4	62.4 ± 1.44	2.31	104
	5	63.5 ± 1.93	3.04	105
MQC2	1	261 ± 19.8	7.58	104
	2	262 ± 3.39	1.29	105
	3	263 ± 17.4	6.61	105
	4	250 ± 13.4	5.38	99.5
	5	255 ± 2.11	0.83	102
HQC	1	417 ± 11.1	2.65	93.1
	2	410 ± 4.03	0.98	91.1
	3	414 ± 7.50	1.81	92.4
	4	414 ± 14.8	3.57	92.3
	5	402 ± 6.83	1.70	89.6
Inter–day variations (30 replicates at each concentration)				
LLOQ		0.111 ± 0.007	6.02	106
LQC		0.31 ± 0.02	6.05	104
MQC1		63.8 ± 1.82	2.85	106
MQC2		258 ± 13.5	5.22	103
HQC		411 ± 10.4	2.54	91.8

Spiked concentrations of LLOQ QC, LQC, MQC1, MQC2 and HQC are 0.105, 0.30, 60.2, 251 and 448 ng/mL, respectively.

Table 3
Stability data for riluzole in plasma (*n*=6)

Stability test	QC (spiked concentration, ng/mL)	Mean±SD (ng/mL)	Accuracy/ Stability (%)	Precision (%)
Aautosampler stability (at 15°C for 72 h)	0.30 448	0.30 ± 0.00 396 ± 2.52	98.2 88.4	0.82 0.64
Wet extract stability (at 2–8°C for 68 h)	0.30 448	0.28 ± 0.03 420 ± 5.36	94.5 93.7	10.8 1.28
Bench top stability (20 h at room temperature)	0.30 448	0.32 ± 0.02 404 ± 3.73	105 90.3	5.10 0.92
Freeze–thaw stability (four cycles)	0.30 448	0.28 ± 0.00 411 ± 23.1	92.5 91.8	0.44 5.61
Reinjection stability (45 h)	0.30 448	0.32 ± 0.02 411 ± 2.67	106 91.8	5.36 0.65
Long–term Stability (at –70°C for 90 days)	0.30 448	0.31 ± 0.01 406 ± 9.36	103 90.6	4.52 2.31
Short–term Stability (at –20°C for 8 days)	0.30 448	0.30 ± 0.00 398 ± 2.69	98.2 88.7	1.49 0.68

Table 4
Incurred samples re–analysis data of riluzole.

Sample	Initial conc. (ng/mL)	Re–assay conc. (ng/mL)	Difference [a] (%)
1	168	182	–8.26
2	1.13	1.03	9.09
3	207	210	–1.60
4	0.43	0.43	–1.40
5	184	182	1.11
6	1.07	0.95	12.4
7	126	117	7.56
8	0.93	1.06	–13.8
9	143	132	7.99
10	0.52	0.56	–8.3
11	160	153	4.73
12	1.68	1.49	11.8

[a] Expressed as [(initial conc.−re–assay conc.)/average]×100%.

Legends to figures:

Figure 1. Product ion mass spectra of $[M+H]^+$ of riluzole.

Figure 2. Typical MRM chromatograms of riluzole (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C).

Figure 3. MRM chromatograms resulting from the analysis of subject blank plasma sample (A) and 5 h subject plasma sample (B), after the administration of a 50 mg oral single dose of riluzole tablet. The sample concentration was determined to be 57.1 ng/mL.

Figure 4. Mean plasma concentration–time profile of riluzole in human plasma following oral dosing of riluzole (50 mg tablet) to healthy volunteers (n=6).

Figure. 1

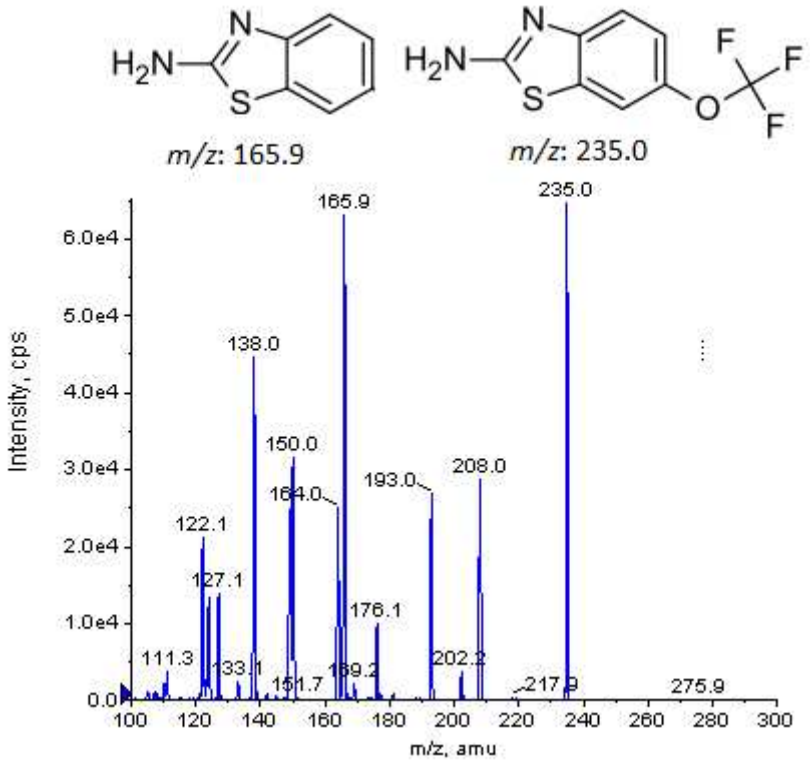

Figure. 2

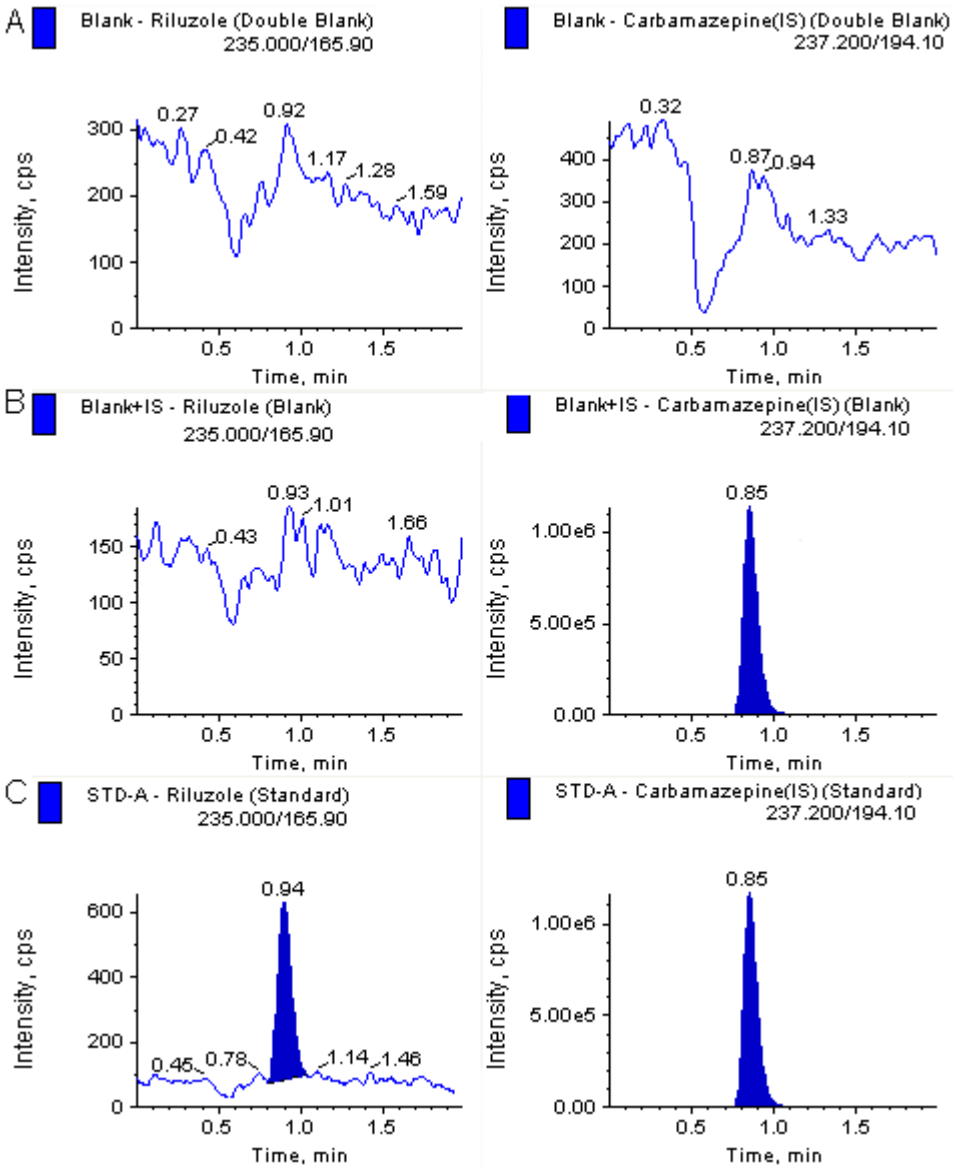

Figure. 3

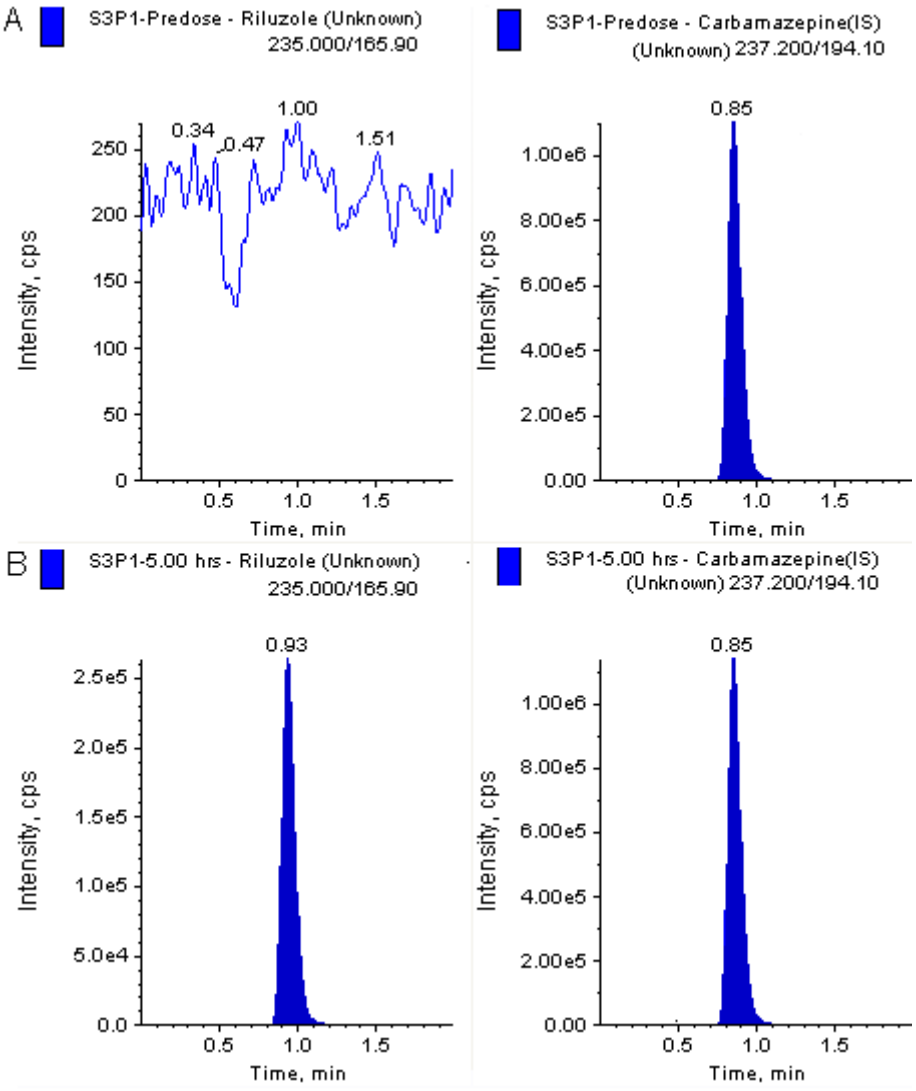

Figure. 4