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HPTLC Method Development and Validation of Riluzole in Bulk and Pharmaceutical Dosage Form

J. Saminathan* and T. Vetrichelvan

Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur, Kanchipuram Dist, Tamilnadu, India-603 319

Abstract

Riluzole is used in the treatment for amyotrophic lateral sclerosis (ALS). The present work describes simple, precise and accurate HPTLC method for the determination of riluzole in bulk and its dosage form. Quantification of riluzole was carried out with Aluminum packed silica gel 60 F 254 precoated TLC plates as a stationary phase using a mixture of mobile phase consists of hexane: ethyl acetate in the ratio 1:1 v/v and in the absorbance/ reflectance mode at 222 nm using a CAMAG TLC scanner 3 with winCATS software version 1.4.3. The *Rf* value of riluzole was found to be 0.34 (±0.02). The proposed method has permitted the quantification of riluzole over the linearity range of 200-1000 ng/spot and its percentage recovery was found to 99.17%. The intraday and interday precisions were found to be 1.26% and 1.40%, respectively. The limit of detection and the limit of quantification were found to be 18 ng/spot and 54 ng/spot respectively. The Coefficient of determination (r^2) was 0.9992. The regression equation was found to be Y = 9.8542C + 276.09.The accuracy and reliability of the proposed method was ascertained by evaluation various validation parameters like linearity, precision, accuracy and specificity according to ICH guidelines. The proposed method was analysed with more formulation units on a single plate and provided a faster and cost effective quality control tool for routine analysis of riluzole in bulk and its dosage form. The excipients in the commercial tablet preparation did n't interfere in the method.

Keywords: Riluzole, ALS, HPTLC, densitometric evaluation, ICH guidelines, method development and validation

*Corresponding author: Mobile phone: 93455 16604

E-mail: swamilingam@gmail.com

1. Introduction

Riluzole (RZ) is a member of the benzothiazole class [1]. Chemically, riluzole (Figure 1) is 2-amino-6-(trifluoromethoxy) benzothiazole. Its molecular formula is $C_8H_5F_3N_2OS$ and its molecular weight is 234.2. Rilutek® (Sanofi Aventis) was obtained commercially with the labeled amount of 50 mg of RZ. Literature survey revealed that various methods have been reported for the determination of RZ in pharmaceutical preparations, rat brain, mouse plasma, serum including spectrophotometry [2], high performance liquid chromatography (HPLC) [3–10], gas-liquid chromatography (GC) [11] and high performance thin liquid chromatography (HPTLC) [12]. Most of the methods reported are highly sophisticated, costly, time consuming and require special sample preparation. The present study illustrates development and validationof a simple, accurate, precise and specific HPTLC method for the estimation of riluzole in bulk and in tablet dosage forms [13]. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution.

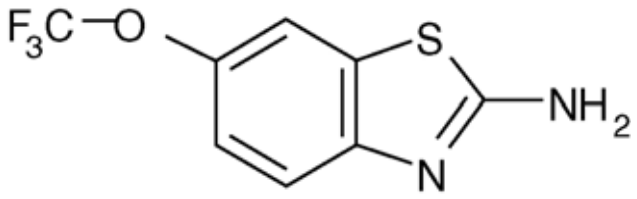


Figure 1 Chemical structure of riluzole

2. Materials and Methods

2.1 Instrumentation

The CAMAG HPTLC system (Muttenz, Switzerland) consisted of CAMAG TLC Scanner 3 with Linomat IV applicator fitted with Hamilton syringe (100µl) controlled by winCATS software version 1.4.3. Autosprayer connected to a nitrogen cylinder, a twin trough chamber (20×10 cm), a derivatization chamber, plate heater were used. Aluminum packed silica Gel 60 F_{254} precoated TLC plates (10×10 cm, layer thickness 0.2 mm (E. Merck KGaA, Mumbai) was used as stationary phase. TLC plates were prewashed twice with 10mL of methanol and activated at 60°C for 5min prior to sample application. Densitometric analysis was carried out using a TLC scanner 3 with winCATS software 1.4.3. Dissolution of the compounds was enhanced by sonication on a Shimadzu sonicator and REMI centrifuge.

2.2 Reagents and Materials

RZ pure powder was obtained as a sample from Watson Pharma (India) with 99.9% purity. Tablet formulation, Rilutek® (Sanofi Aventis) was obtained commercially with the labeled amount of 50 mg of RZ. Double distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

2.2.1 Preparation of RZ Standard Stock Solution

A working standard of riluzole of ~ 5 mg was accurately weighed and transferred into 25 ml volumetric flask. A volume of methanol (~10 ml) was added and sonicated for about 10 min. The volume was finally made up to 25ml with methanol.

2.3 HPTLC Method and Chromatographic Conditions

2.3.1 Sample Application

The standard and formulation samples of RZ were spotted on Aluminum packed silica Gel 60 F_{254} precoated TLC plates in the form of narrow bands of 6 mm lengths, with 10 mm from the left margin and with 9mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 600 nL/s**.**

2.3.2 Mobile Phase and Migration

Plates were developed using mobile phase consisting of hexane: ethyl acetate in the ratio of 1:1 v/v. Linear ascending development was carried out in 20 cm×10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at 25±2°C. Ten milliliters of the mobile phase (5mL in trough containing the plate and 5mL in other trough) was used for each development and allowed to migrate at a distance of 70 mm, which required 10min. After development, the TLC plates were dried completely.

2.3.3 Densitometric Analysis and Quantitation Procedure

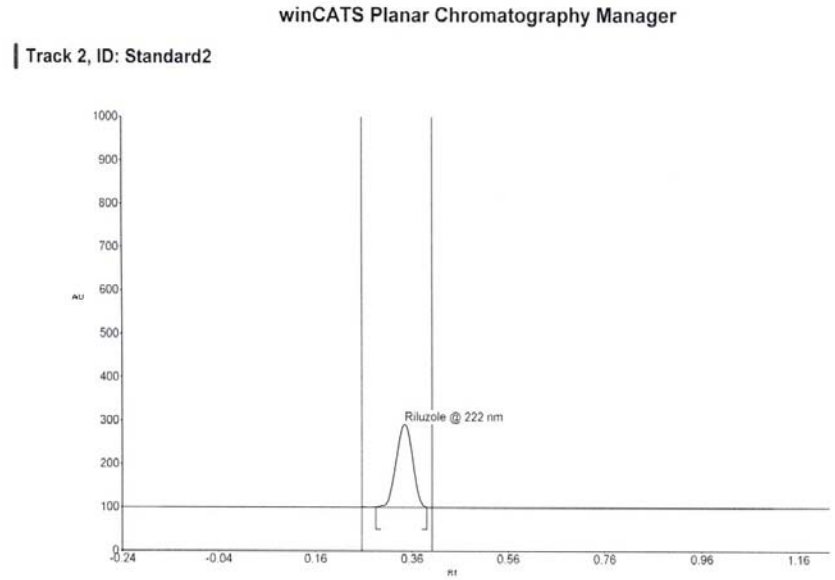
Densitometric scanning was performed on *CAMAG* TLC scanner 3 in absorbance mode and operated by winCATS software version 1.4.3. The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 222 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning speed of 100 nm/s. These are selected as recommended by the CAMAG TLC scanner 3 manual. It covers 70%–90% of the application band length, which in the present case is 6 mm. The monochromator bandwidth was set at 20 nm. Concentrations of compound chromatographed were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using linear regression equation.

3. Method Validation

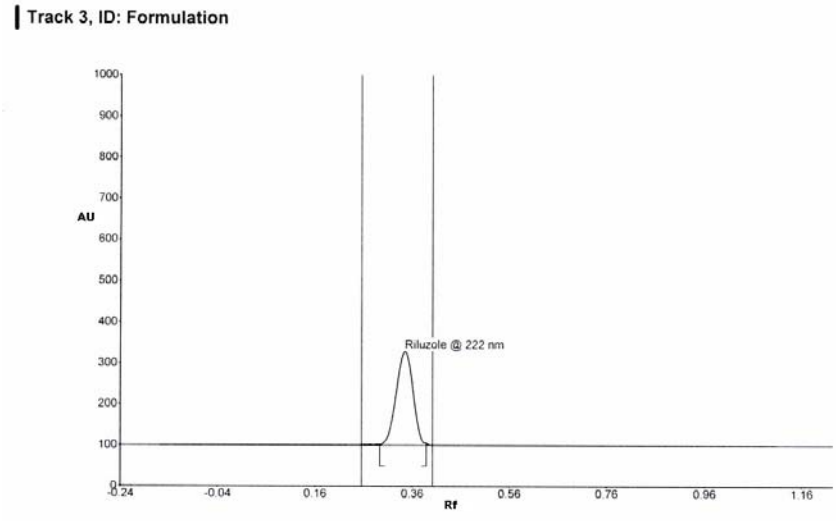
Validation of the developed HPTLC method of riluzole was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness [13].

3.1 Specificity

The specificity of the developed method was established analyzing the sample solutions containing RZ from marketed tablets. The spot for RZ in the sample was confirmed by comparing retardation factor (*Rf*) values of the spot with that of the standard shown in Figure 2.



2a (standard)



2b (sample)

Figure 2 A typical densitogram of riluzole

3.2 Sensitivity

Sensitivity of the developed method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). Noise was determined by scanning blank spot (methanol) six times. Series of concentrations of drug solutions (200–1000 ng/spot) were applied on plate and determined for LOD and LOQ. LOD was calculated as 3 times the noise level, and LOQ was calculated as 10 times the noise level. LOD and LOQ were experimentally verified by diluting the known concentrations of RZ until the average responses were approximately 3–10 times the standard deviation (SD) of the responses for six replicate determinations.

3.3 Linearity and Calibration Curve

Aliquots (1, 2, 3, 4, 5 µl) of standard solution of riluzole were spotted on Aluminum packed silica Gel 60 F_{254} precoated TLC plates using semi automatic spotter under nitrogen stream. The plate was dried in oven and developed up to 72 mm at constant temperature with a mixture of hexane: ethyl acetate in the ratio 1:1 v/v as mobile phase in a CAMAG twin trough chamber which was previously saturated with mobile phase for about 30 min. The plate was removed from the chamber and dried in an oven. Photometric measurements were performed at 222 nm in absorbance/reflectance mode with the CAMAG TLC scanner 3 using winCATS planar chromatography software version 1.4.3 incorporating track optimizing option. The standard plot of riluzole was established by plotting the peak area Vs concentration (ng/spot) corresponding to each spot. Linearity of the method was evaluated by constructing calibration curves at six concentration levels. Calibration curves were plotted over a concentration range of 200–1000 ng/spot as shown in Figure 3. Accuracy of the method was evaluated by carrying the recovery study at three levels. Recovery experiments were performed by adding three different amounts of standard drug, that is, 25%, 50%, and 75% of the drug, to the preanalyzed formulations, solution and the resultant was reanalyzed six times as shown in Table 1. Precision was evaluated in terms of intraday and interday precisions. Intraday precision was determined by analyzing sample solutions of RZ from formulations at three levels covering low, medium, and high concentrations of three times on the same day. Interday precision was determined by analyzing sample solutions of RZ at three levels covering low, medium, and high concentrations over a period of three days (n = 3). The peak areas obtained were used to calculate mean and % RSD (relative standard deviation) values. Repeatability of measurement of peak area was determined by analyzing different amount of RZ samples covering low, medium, and high ranges of the calibration curve three times without changing the position of plate. Repeatability of sample application was assessed by spotting RZ samples covering similar range of calibration curve six times and analyzing them once. By introducing small changes in mobile phase composition, its volume, chamber saturation time and slight change in the solvent migration distance, the effects on the results were examined. Robustness of the method was determined in triplicate at a concentration level of 600 ng/spot and the mean and % RSD of peak area shown in Table 2.

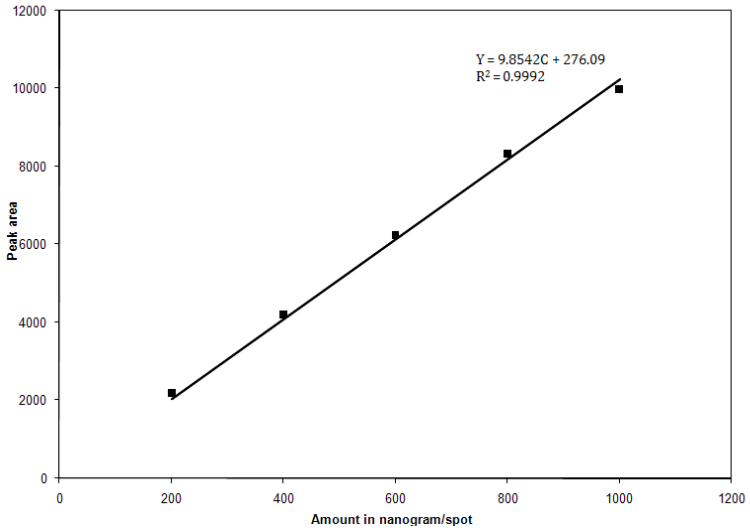


Figure 3 Linearity curve of riluzole

Table 1 Recovery studies of the method

Sample no.	Amount of drug added (%)	Theoretical content (ng)	recovery±SD*	% RSD
1	25	150	99.71±0.24	0.64
2	50	300	100.18±0.37	0.79
3	75	450	100.83±0.43	0.91

*****Values are recovery ± SD of three determinations

Table 2 Robustness of the method

Sample no.	PARAMETER	Amount of riluzole spotted (ng/spot)	Amount* of riluzole detected (ng/spot)	MEAN %RSD
1	Mobile phase composition 5.5:4.5	600	598.22 ± 1.40	2.02
2	Mobile phase composition 6:4	600	601.18 ± 1.35	2.36
3	Chamber saturation time:15min	600	598.93 ± 1.41	1.91
4	Chamber saturation time:25min	600	600.64 ± 1.23	2.03
5	Solvent migration distance:68mm	600	597.79 ± 3.07	1.67
6	Solvent migration distance:72mm	600	599.07 ± 1.90	1.75

* Each value is the mean ± S.D of six determinations

3.4 Estimation of Riluzole in Marketed Tablet Formulation

Twenty tablets were accurately weighed and finely powdered. The powder which is equivalent to 50 mg of riluzole was weighed, mixed with 25 ml of methanol and sonicated for 15 min. The solution of tablet was filtered through Whatmann filter paper No. 41 and the residue was thoroughly washed with methanol. From this stock solution 1.5 ml was taken and the volume made up to 10mL. Two microliters of sample solution equivalent to 600 ng of riluzole was applied on a TLC plate under a nitrogen stream using a semi automatic spotter. The amount of riluzole present in the sample solution was determined by fitting the area values of peaks corresponding to riluzole into the equation of the line representing the calibration curve of riluzole.

4. Results and Discussion

To develop HPTLC method of RZ for routine analysis, selection of mobile phase was carried out on the basis of polarity. A solvent system that would give dense and compact spots with appropriate and significantly different *Rf* value for RZ was desired. Various solvent systems such as acetone:methanol, methanol:chloroform, methanol:toluene, methanol:ethylacetate, toluene:ethyl acetate, toluene:ethyl acetate-methanol, hexane:ethyl acetate, hexane:acetone, toluene:acetonitrile, and toluene:acetonitrile:glacial acetic acid were evaluated in different proportions. Among these, the solvent system comprising hexane: ethyl acetate (1:1 v/v) gave good elution of RZ with a *Rf* value of 0.34 (±0.02). It was also observed that chamber saturation time and solvent migration distance are crucial in chromatographic separation as chamber saturation time of less than 15min and solvent migration distances greater than 70 mm resulted in diffusion of analyte spot. Therefore, hexane: ethyl acetate solvent system in 1:1 v/v proportion with chamber saturation time of 30 min at 25°C and solvent migration distance of 70 mm was used as mobile phase. These chromatographic conditions produced a well defined compact spot of RZ with optimum migration at $Rf = 0.34 \pm 0.02$ (Figure 4). It also gave a good resolution of analyte from excipients used in solutions and marketed tablet formulations. Under the experimental conditions employed, the lowest amount of drug that could be detected was found to be 18 ng/spot and the lowest amount of drug that could be quantified was found to be 54 ng/spot, with RSD <5%. Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of sample matrix. RZ was separated from excipients with an *Rf* of 0.34 ± 0.02. In addition, there was no interference from excipients, present in commercial formulation, thereby confirming specificity of method. Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Method was found to be linear in a concentration range of 200–1000 ng/spot ($n = 5$) with respect to peak area. The regression data as shown in Table 3 reveal a good linear relationship over the concentration range studied demonstrating its suitability for analysis. The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions as shown in Table 4. Intraday precision refer to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment is shown in Table 5 whereas interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days is shown in Table 6. Ruggedness study was performed by different analysts. The results obtained are shown in Table 7. In all instances, % RSD values were less than 5% confirming the precision of the method. Two-microliter aliquots of samples containing 600, 800, and 1000 ng of RZ were analyzed according to the proposed method. In order to control scanner parameters, that is, repeatability of measurement of peak area, one spot was analyzed without changing position of plate ($n = 3$). By spotting and analyzing the same amount several times ($n = 3$), precision of automatic spotting device was evaluated and % RSD was consistently

less than 5% (Tables 4-6), which was well below the instrumental specifications, ensuring repeatability of developed method as well as proper functioning of the HPTLC system. The low values of % RSD (Table 8) obtained after introducing small deliberate changes in the developed HPTLC method confirmed the robustness of the method.

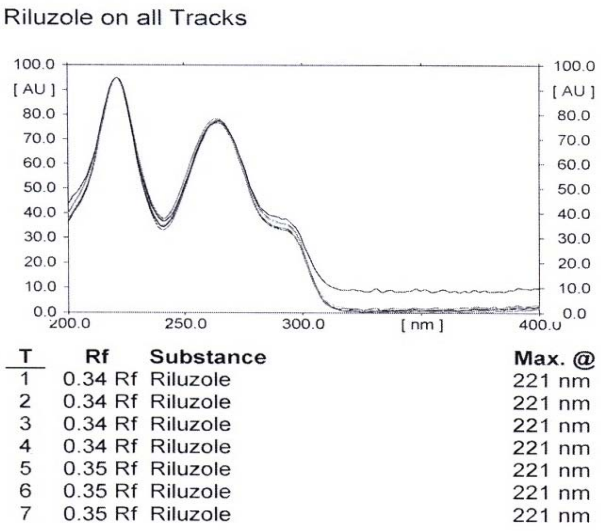


Figure 4 HPTLC chromatogram of riluzole

Table 3 Linearity of the method

Sample No.	Amount in ng/spot	Peak area
1	200	2189.16
2	400	4203.55
3	600	6240.68
4	800	8329.40
5	1000	9980.50

Table 4 Precision of the method

Sample no.	Concentration (ng/spot)	Peak area
1	600	6240.22
2	600	6310.38
3	600	6338.44
4	600	6320.45
5	600	6320.52
6	600	6220.68

Mean peak area ± SD: 6291.11± 48.95; %RSD – 0.77

Table 5 Intra-day precision of the method

Sample no.	Concentration (ng/spot)	Area	Mean*	S.D	% RSD
1 2 3	600	6285.52 6262.48 6320.22	6289.40	29.06	0.46
1 2 3	800	8349.45 8319.52 8285.29	8318.08	32.10	0.38
1 2 3	1000	9942.45 9970.24 9920.32	9944.33	25.01	0.25

*Mean of nine determinations (3 replicates at 3 concentration levels)

Table 6 Inter-day precision of the method

Sample.no	Concentration (ng/spot)	Area	Mean*	S.D	% RSD
1 2 3	600	6242.58 6322.82 6349.52	6304.97	55.65	0.88
1 2 3	800	8379.72 8339.45 8375.12	8364.76	22.04	0.26
1 2 3	1000	9942.75 9945.24 9915.32	9934.43	16.60	0.16

*Mean of nine determinations (3 replicates at 3 concentration levels)

Table 7 Ruggedness of the method

Variable	% recovery[1](*n* = *6*)	% RSD[1]
Analyst 1	100.60±0.43	0.97
Analyst 2	99.34±0.73	1.02

[1]Average for two amounts: 200 and 400ng/band.

Table 8 Content of riluzole in various formulations

Formulation	Label claim (mg/tablet)	Amount found* (mg/tablet)	% RSD
Rilutek™ Tablets	50mg	49.88±0.27	1.10

*mean of six determinations (n=6)

5. Conclusions

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of riluzole in bulk and pharmaceutical formulations.

6. Acknowledgements

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