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Validation of UV Spectrophotometric Method for Determination of Riluzole in Pharmaceutical Dosage Form

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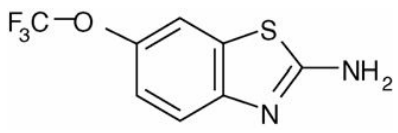
Abstract: A simple and reproducible method was developed for the assay of riluzole in tablets. The excipients in the commercial tablet preparation did not interfere with the assay. Various methods for analysis of the same are available but are time consuming and expensive. Here we have developed a new, precise and simple validated UV spectrophotometric method for determination of riluzole from bulk and tablet formulation. The drug obeyed the Beer's law and showed the regression line (Y=0.0458x+0.0032) and correlation coefficient 0.9999. It showed absorption maxima at 263.5 nm in methanol. The linearity was observed between 2.0 – 20µg/mL. The molar absorptivity was found to be 10814.284. The results of analysis were validated by recovery studies. The % recovery was found to be 99.2 – 100.32%. Six triplicate analyses of solutions containing six different concentrations of the examined drug were carried out. The proposed method was applied to the determination of the examined drug in coated tablet and the results demonstrated that the method is equally accurate, precise and reproducible as the official methods.
Keywords:Amyotrophic lateral sclerosis (ALS), Riluzole, Method validation, Spectrophotometry.

Introduction

Riluzole[1] chemically it is 2-amino-6-(trifluoromethoxy) benzothiazole. It is used in the current treatment for amyotrophic lateral sclerosis (ALS)[2], a chronic neurodegenerative disease causing progressive motor weakness resulting from selective motor neuron cell death. No official method is available for the assay for riluzole and its formulations. Some methods such as on high-performance liquid chromatography with ultraviolet detection (HPLC-UV)[3] or coupled with tandem mass spectrometry (LC/MS/MS),[4] recent method based on high-performance liquid chromatography with ultraviolet detection, LC5 and HPLC-UV[6] methods have been described for riluzole. Riluzole (fig.1) was demonstrated to modulate the anti-glutamatergic activity through glutamate and sodium receptors. But no method for riluzole as raw material and in pharmaceutical formulations had been previously described. The aim of this work was to develop a simple and reproducible spectrophotometric procedure for the determination of riluzole in raw material and coated tablets.

Fig.1: Structure of riluzole



2. Experimental

2.1. Chemicals

The Riluzole reference substance (assigned purity 99.6%) and coated tablets containing riluzole was supplied by sanofi-aventis (Mumbai). The Rilutek tablets (sanofi-aventis) where claimed to contain 50 mg (as anhydrous base) of drug and the following

excipients: anhydrous dibasic calcium phosphate, microcrystalline cellulose, anhydrous colloidal silica, magnesium stearate, croscarmellose sodium, hypromellose, polyethylene glycol 6000, titanium dioxide. Riluzole is available as 50 mg tablets and it is well absorbed with average oral bioavailability of 60%.

Reagents and solvents
Methanol analytical grade.

Instrumentation and conditions
Spectral and absorbance measurements were made with a Shimadzu UV-VIS detector with 10 mm quartz cells at 263.5 nm. The solutions were prepared in Methanol.

Procedure:

Determination of λ max:
Weighed amount of riluzole was dissolved in methanol to obtain a 100µg/mL stock solution. Absorption maxima was studied by diluting the above solution to 20µg/mL and scanned from 200 – 400nm to obtain a maximum absorbance at 263.5nm in fig.2.

Standard Stock Solution:
A stock solution containing 200 µg/mL of pure drug was prepared by dissolving 20mg of riluzole in sufficient methanol to produce 100 mL solution in a volumetric flask

Linearity and Calibration:
The aliquots standard stock solution was diluted serially with sufficient methanol to obtain the various concentration ranges of 2.0 – 20 µg/mL in the overlain spectra in fig.3**.** A calibration curve for riluzole was obtained by measuring the absorbance at the λmax of 263.5 nm in fig.4. Statistical parameters like the slope, intercept, coefficient of correlation, Beer's law limit, Molar Absorptivity, Sandell's sensitivity were determined

Assay of Riluzole in tablets:
To analyze the concentration of riluzole tablets, twenty tablets were weighed to obtain the average tablet weight. The tablets were ground up and 20 mg were transferred to a 100 mL volumetric flask; with methanol were added and the flask was shaken for 10 minutes by mechanical shaker followed by addition of methanol to volume (final concentration of 200 µg.mL–1). Aliquots of 3 mL of this solution were transferred to a 50 mL volumetric flask and methanol was added to volume to give an estimated concentration of 12 µg.mL–1.This solution was prepared six times and the absorbance of each solution was determined at 263.5nm using methanol as blank. All determinations were conducted in triplicate.

Method validation:
The accuracy and precision of the assay, as well linearity of the calibration curve, were determined (Fig.3)[8,9]. Having established the quantitative relationships between the parameters studied, and knowing the predictive performance of their association model, a linear simple regression by the least squares method was applied. The statistical analysis was calculated by ANOVA. The statistical recovery was determined by adding known amounts of riluzole reference standard to the samples at the beginning of the process. 20 mg equivalent powdered tablet added with 2.0, 3.0 ,4.0 mL of riluzole standard solution (3mg.mL–1) and a aliquot of 3 of this solution were transferred to 50 mL volumetric flasks, respectively, R1, R2 and R3 (Table 3). The percentage recovery for added riluzole standard was calculated using the equation proposed by AOAC [8].

Fig.2 Absorption maximum of riluzole in methanol

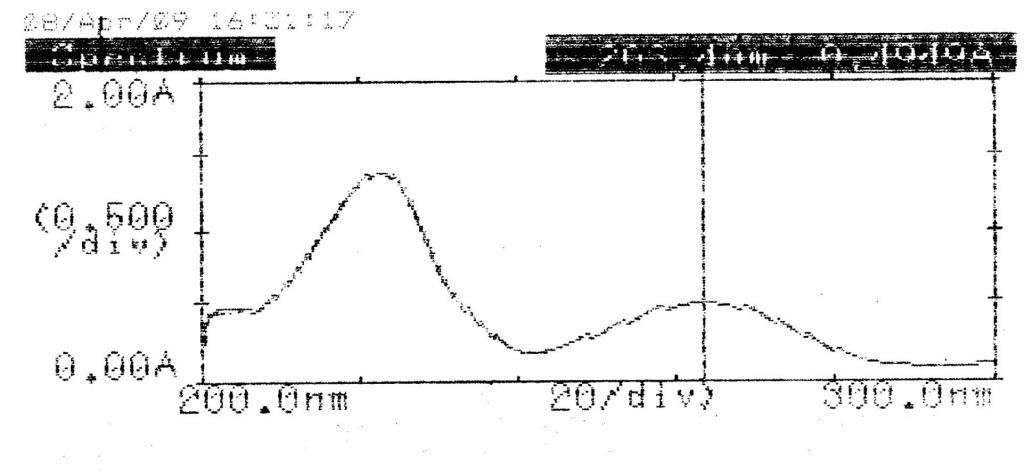
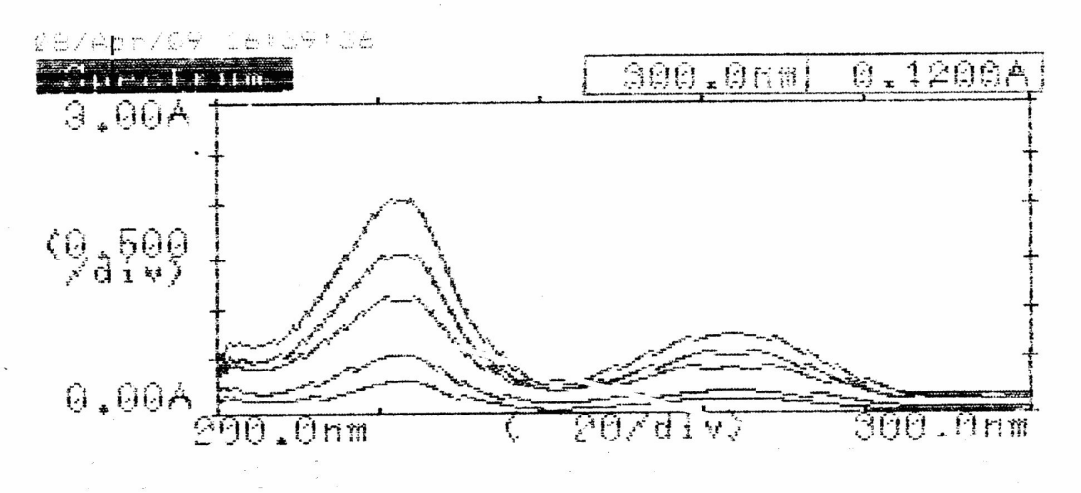


Fig.3 overlain spectra of riluzole in various concentrations



Accuracy:
To assess the accuracy[9] of the proposed method, recovery studies were carried out three different levels i.e. 50%, 75% and 100%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded. The % recovery was then calculated as % Recovery = [(A – B) / C] x 100, Where A is total amount of drug estimated; B is amount of drug found on preanalyzed basis; C is amount of pure drug added to formulation (Table 3).

Precision:
Precision[10] of the method is studied as repeatability, intra-day and interday precision. Repeatability was determined by analyzing rilutek (25µg/mL) for six times (Table 4). Intra-day precision was determined by analyzing the 15, 20 and 25µg/mL of riluzole for three times in the same day. Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days (Table 5). In intermediate precision study, % R.S.D values were not more than 1.0 % in all the cases.

Ruggedness:
Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions (Table 6)

Limit of detection and Limit of quantitation:
Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by using the formula based on the standard deviation of the response and the slope. Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated by using the equations LOD = 3 × s/S and LOQ = 10 × s/S, where s is standard deviation of intercept, S is the slope of the line. (Table 1)

Table-1: Quantitative parameters for the determination of Riluzole in dosage form.

Parameters	Results
λmax (nm)	263.5nm
Molar absorptivity (lit/mole/cm)	10817.84
Beer's law (µg/mL)	2-20µg/ml
Regression equation	Y=0.0458x+0.0032
Slope(a)	0.0458
Intercept(b)	0.0032
Correlation coefficient (r2)	0.99992
Sandell's sensitivity (µg/sq.cm/0.001)	0.02180
LOD (µg/mL)	0.5682
LOQ (µg/mL)	1.7221
n	6

Table-2: Determination of Riluzole in dosage form.

S.no	Dosage form	Experimental amount (mg)	% Percentage purity
1	50mg	49.8770	99.75
2	50mg	50.0204	100.20
3	50mg	50.0166	100.16
4	50mg	49.9342	99.86
5	50mg	49.6767	99.35
6	50mg	49.5674	99.13

*Each value is the mean of three analyses

Table-3: Experimental values obtained in the recovery study for Riluzole in dosage form

S.no	Amount of reference(µg/ml)		% Recovery
	Added	recovered	
R1	6.0087	6.044	99.59
R2	9.0588	9.0742	100.17
R3	12.0283	12.0556	100.22

*Each value is the mean of three analysis

Table 4: Results of repeatability studies

Label claim	Amount taken (µg/ml)	Amount found %	% RSD
50 mg	12	99.9 ± 0.34	0.34

*Mean of six observations

Table 5: Intra-day and Inter-day precision for 50mg

S.no	Concentration	intraday	% RSD	interday	% RSD
1	12	11.97 ± 0.02	0.31	11.87 ± 0.03	0.41
2	15	15.02 ± 0.07	0.62	15.01±0.09	0.80
3	18	18.08±0.23	1.45	17.81±0.21	1.35

*Mean of three observations

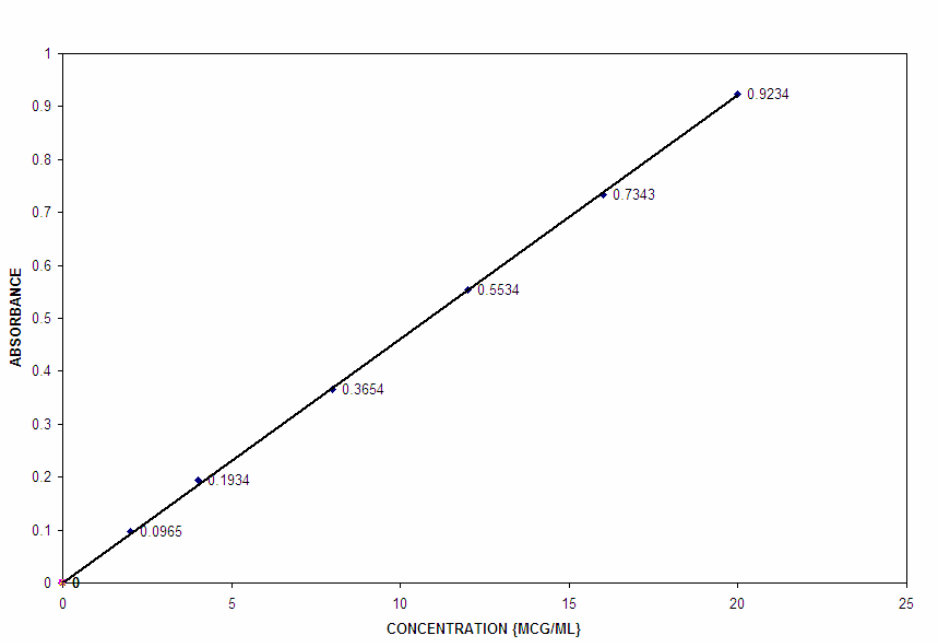
Table 6: Results of Ruggedness study

Label claim	Analyst 1	% RSD	Analyst 2	% RSD
50 mg	99.48 ± 0.62	0.65	99.46 ± 0.73	0.74

Results and Discussion:

The development of spectrophotometric methods for the determination of drugs has increased considerable in recent years because of their importance in pharmaceutical analysis. The calibration curve for riluzole was obtained plotting the peak area versus concentration. Linearity was found to be in the range of 2.0 - 20 µg.mL–1 with significantly high value of correlation coefficient r2 = 0.9999; the representative equation was y = 0.0458x+0.0032. The quantitative parameters for determination of riluzole in pharmaceutical dosage form are listed in Table 1.The coefficient of variation (CV) on the basis of the absorbance's for six triplicate measurements found to be between 0.1297 and 1.16%. Riluzole tablets (50 mg) were analyzed and the results obtained can be seen in Table 3. The percentage of gotten pureness was of 99.27% and the coefficient of variation of 0.45%. The assays were validated by means of the analysis of variance, as described in official literature.[7,8] This developed method presented no parallelism deviation and no linearity deviation ($P < 0.05$). The precision and recovery of the assay were demonstrated. The accuracy express the agreement between the accepted value and the value found. The recoveries obtained showed that a high accuracy of the presented method.

Fig.4 Calibration curve of riluzole at 263.5nm



Conclusion:

The obtained and statistical parameters for determination of riluzole in raw material and coated tablets demonstrate that the proposed UV spectrophotometric method by is simple, accurate, fast and precise. The method showed acceptable linearity and recovery. The proposed method is highly sensitive; therefore it could de used easily for the routine analysis of pure drugs and their formulations.

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