

**RP-HPLC and HPTLC Methods for the Estimation of  
Riluzole in Tablet Dosage form**

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**ABSTRACT**

Two simple, specific, accurate and precise methods, namely, Reverse Phase High Performance Liquid Chromatography and high performance thin layer chromatography were developed for estimation of Riluzole in tablet dosage form. For the HPLC method, Promosil C-18, 5  $\mu$ m column consisting of 200  $\times$  4.6 mm i.d in isocratic mode, with mobile phase containing 25 mM  $\text{KH}_2\text{PO}_4$  buffer (pH 2.8) : acetonitrile: (40:60 v/v) was used. The flow rate was 1 ml/min and effluent was monitored at 265 nm. The retention time was found to be 6.7 min. For the high performance thin layer chromatographic method a Camag system comprising of Linnomat V automatic sample applicator, Hamilton syringe, Camag TLC Scanner-3, Camag WinCATs software with stationary phase precoated silica gel 60F<sub>254</sub> and mobile phase consisting of chloroform : ethyl acetate : methanol (4:5.9:0.1 v/v/v) were used. The detection of spot was carried out at 265 nm. The  $R_f$  value was found to be 0.75. The methods were validated in terms of linearity, accuracy and precision. The linearity curves were found to be linear over 35-115  $\mu$ g/ml for high performance thin layer chromatography and 100-900 ng/spot for high performance thin layer chromatography. The proposed methods were successfully used for estimation of Riluzole in tablet dosage form.

**KEY WORDS:** Riluzole, HPLC, HPTLC, validation, analysis

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**INTRODUCTION**

The glutamate antagonist Riluzole or 2-amino-6-trifluoromethoxy-benzothiazole is the only drug proven effective for the treatment of patients with amyotrophic lateral sclerosis (ALS), a neurodegenerative disease. The methods<sup>3-7</sup> for analysis of Riluzole in biological fluids is reported in the literature. The present investigation describes two precise, accurate and specific reverse Phase high performance liquid chromatography (RP-HPLC) and high performance thin layer chromatography (HPTLC) methods for the estimation of Riluzole in tablet formulation.

The entire reagents used were of HPLC and anal grades. Reference standard of riluzole was obtained from Sun Pharma Ltd, Mumbai. Tablets of one brand, Rilutor (50mg) of Sun pharma was taken as gift sample from the same as it is not available in the market. A standard stock solution of riluzole (1 mg/ml) was prepared by dissolving 25 mg of the drug in 25 ml of methanol. For HPLC method, working standard solution (500 $\mu$ g/ml) was obtained from stock solution by dilution with mobile phase and for HPTLC method, working standard solution (100  $\mu$ g/ml) was obtained from stock solution by dilution with methanol.

HPLC system including Waters 2997 PDA detector, empower software, Promosil C18 (250 x 4.6 x 5) column, In-line degasser AF with 717 Plus autosampler was used. Mobile phase was prepared by mixing 25 mM  $\text{KH}_2\text{PO}_4$  (pH2.8) & acetonitrile in proportion of 40:60 v/v, respectively.

Linearity of the method was investigated by serially diluting the stock solution to give a concentration range of 35 to 115  $\mu\text{g/ml}$ . The flow rate was maintained at 1ml/min. Temperature of the column was kept ambient and the effluent was monitored at 265 nm. Calibration curve was constructed by plotting concentration against peak area.

A Camag HPTLC system comprising of Linomat V automatic sample applicator, Hamilton syringe, Camage TLC scanner-3, Camag WinCATs software, Camag twin trough chamber and as stationary phase, precoated silica gel 60F254 were used. TLC plates were pre-washed with methanol. Activation of plates was done in an oven at 50<sup>0</sup>C for 5 min. The chromatographic conditions maintained were precoated silica gel 60F254 aluminum sheets as stationary phase, chloroform : ethyl acetate : methanol (4 : 5.9 : 0.1 v/v/v) as mobile phase, chamber saturation time of 30 min, migration distance allowed was 75 mm, scanning was done at 265 nm keeping the slit dimension at 6 x 0.45 mm. A deuterium lamp provided the source of radiation

Aliquots (1, 2, 3, 4, 5, 6, 7, 8 & 9  $\mu\text{L}$ ) of standard solution (100  $\mu\text{g/ml}$ ) of Riluzole were applied on the precoated silica gel 60F<sub>254</sub> TLC plate. The TLC plate was dried, developed and analyzed photometrically as described earlier.

Calibration curve was constructed by plotting peak area against concentration.

Assay of the marketed tablets with brand name Rilutor (50 mg) was performed. Twenty tablets of the above brand were separately weighed and powdered. The powder equivalent to 25 mg of riluzole was dissolved in methanol to obtain 1 mg/ml, and was ultrasonicated and filtered through 0.45 micron membrane filter. The solution was further diluted with the mobile phase for HPLC and with methanol for HPTLC, and subjected for HPLC and HPTLC analysis as described earlier. From the peak area of riluzole, the amount of drug in sample was computed.

To optimize the HPLC parameters, several mobile phase composition were tried. Satisfactory peak symmetry was obtained with mobile phase consisting of 25 mM  $\text{KH}_2\text{PO}_4$  (pH was adjusted to 2.8 with 10% v/v o-phosphoric acid): acetonitrile (40:60v/v). Quantification was achieved with UV detection at 265 nm based on peak area. The retention time was 6.7 min.

As per the USP XXIII<sup>8</sup>, system suitability tests for HPLC were carried out on freshly prepared standard stock solution of Riluzole and the parameters studied and results obtained with 10  $\mu\text{l}$  injection volumes are summarized in Table 1.

In HPTLC method, several combinations of solvents were tried to accomplish separation. Using solvent system chloroform: ethyl acetate: methanol (4 : 5.1 : 0.1 v/v/v) and precoated silica gel 60F<sub>254</sub> aluminium plate as stationary phase, good separation was attained, where  $R_f$  was found to be at 0.75. The quantification of the drug was carried at

265 nm wavelength. The linear regression data showed a good linear relationship over a concentration range of 35 to 115 µg/ml for HPLC and 100 to 900 ng/spot for HPTLC. The intra-day and inter-day precision were determined by analyzing standard solutions in the concentration of 55, 75 & 95 µg/ml for HPLC and 320, 400 & 480 ng/spot for HPTLC. The intra-day and inter-day results indicate that both methods are precise (Table 2).

Assay results of Rilutor (Sun Pharma) are very close to the label claim. To study accuracy of the developed methods.

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## ACKNOWLEDGEMENTS

The authors thank the Sun Pharma Ltd., Mumbai, for providing the gift sample of the drug and Delhi Institute of Pharmaceutical Sciences & Research, New Delhi, for providing facilities to carry out this work.

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**TABLE: 1** VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	RP-HPLC	HPTLC
Retention time (min)	6.7	-
Rf value	-	0.75
Linearity range	35-115 µg/ml	100-900 ng/spot
Correlation coefficient (r <sup>2</sup> )	0.999	0.9996
Regression equation (y=mx+c)		
Slope (m)	18577	2.8822
Intercept (c)	807.8	142.09
Tailing factor	1	-
Theoretical plates	31920	-

y = peak area, x = Concentration in µg/ml

**TABLE 2:** INTRA-DAY AND INTER-DAY PRECISION STUDY

Concentration		Intra day (% RSD)		Inter day (% RSD)	
HPLC	HPTLC	HPLC	HPTLC	HPLC	HPTLC
µg/ml	ng/spot				
55	320	0.040	0.351	0.012	0.341
75	400	0.006	0.369	0.006	0.355
95	480	0.006	0.296	0.005	0.293

RSD = Relative standard deviation (n=6)

**TABLE 3:** ASSAY RESULTS AND % RECOVERIES FOR RILUZOLE TABLETS

Formulation	Label Claim mg	RP-HPLC method		HPTLC method	
		Amount found* mg	% Assay	Amount found* mg	% Assay
RILUTOR	50	49.995	99.99	50.102	100.204

\*Average of three determinations