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## A stability indicating LC method for riluzole in bulk drugs and pharmaceutical dosage forms

Ravi Kiran Kaja<sup>1,2\*</sup>, K.V.Surendranath<sup>1</sup>, P.Radhakrishnanand<sup>1</sup>, J.Satish<sup>1</sup>, P.V.V Satyanarayana<sup>2</sup>

<sup>1</sup>United States Pharmacopeia-India Private Limited, Research and Development Laboratory, ICICI Knowledge Park, Turkapally, Shameerpet, Hyderabad-500 078, (INDIA)

<sup>2</sup>Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur- 522510, (INDIA)

E-mail : ravikiran.kaja@yahoo.co.in

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### ABSTRACT

A novel, sensitive, stability indicating RP-LC method has been developed for the quantitative determination of riluzole, its related impurities in both bulk drugs and pharmaceutical dosage forms. Efficient chromatographic separation was achieved on a C18 stationary phase with simple mobile phase combination delivered in an isocratic mode and quantitation was carried out using ultraviolet detection at 241 nm. The mobile phase consists of buffer and acetonitrile. Buffer consists of 10 mM ammonium acetate, pH adjusted to 8.5 by using triethylamine and delivered at a flow rate of 1.2 mL min<sup>-1</sup>. In the developed HPLC method the resolution ( $R_s$ ) between Riluzole and its three potential impurities was found to be greater than 2.0. Regression analysis shows an r value (correlation coefficient) of greater than 0.999 for Riluzole and it's all the three impurities. This method was capable to detect all three impurities of Riluzole at a level of 0.0003% with respect to test concentration of 1.0 mg mL<sup>-1</sup> for a 20 $\mu$ L injection volume. The inter and intra day precision values for all three impurities and for Riluzole was found to be within 2.0% RSD. The method has shown good and consistent recoveries for Riluzole in bulk drugs (98.3-101.1%), pharmaceutical dosage forms (101.0-103.1%) and it's all the three impurities (99.7-101.7%). The test solution was found to be stable in diluent for 48 h. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in oxidative conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.95%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness. © 2008 Trade Science Inc. - INDIA

### KEYWORDS

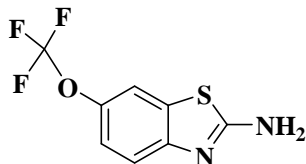
Column liquid chromatography;  
Riluzole;  
Pharmaceutical dosage forms;  
Forced degradation;  
Validation;  
Stability indicating.

### INTRODUCTION

Riluzole is a member of the benzothiazole class. Chemically, Riluzole is 2-amino-6-(trifluoromethoxy) benzothiazole (Figure 1) is an anti-depressant, neuroprotective drug used to treat Amyotrophic Lateral Sclerosis (ALS) or ALS-plus syndrome and Motor

Neurone Disease. It is also used to treat depression in bipolar disorder. The generic name of Riluzole is Rilutek®, preferentially blocks TTX-S sodium channels, which are associated with damaged neurons. This reduces influx of calcium ions and indirectly prevents stimulation of glutamate receptors. The human body naturally produces glutamate, which carries signals to

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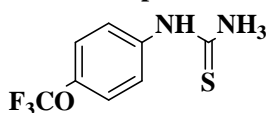


(2-amino-6-(trifluoromethoxy) benzothiazole)

Molecular formula:  $C_8H_5F_3N_2OS$ 

Molecular weight: 234.2

Imp-1

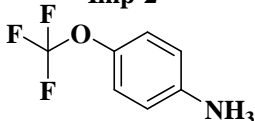


Thiourea[4-(trifluoromethoxy) phenyl]

Molecular Formula:  $C_8H_7F_3N_2OS$ 

Molecular Weight: 177.12

Imp-2

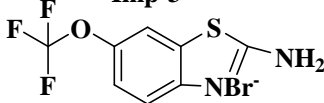


4-Trifluoromethoxy aniline.

Molecular Formula:  $C_7H_6F_3NO$ 

Molecular Weight: 177.12

Imp-3



2-Amino-6-trifluoromethoxy-benzothiazol-3-ium; bromide

Molecular Formula:  $C_8H_5F_3N_2OS.Br$ 

Molecular Weight: 177.12

Imp-4

Figure 1 : Chemical structures and labels of Riluzole and its impurities

the motor neurons. Together with direct glutamate modulating agent Riluzole was effective in treatment-resistant unipolar and bipolar depression<sup>[4-6]</sup>.

Few analytical methods were reported in literature for the quantification of Riluzole in mouse plasma and central nervous system tissues<sup>[7,8]</sup>. Extensive literature survey reveals there is no stability-indicating LC method for quantitative estimation of Riluzole. An ideal stability indicating chromatographic method should estimate the drug be able to resolve from its potential impurities and degradation products. The present drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to separation of degradation products and hence supporting the suitability of

the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated [9-11].

Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for the determination of Riluzole and all the three impurities in bulk drug samples and in pharmaceutical dosage forms along with method validation as per ICH norms. The stability tests were also performed on both drug substances and drug product as per ICH norms.

## EXPERIMENTAL

## Chemicals

Samples of Riluzole and its related impurities were received from USP-Inida private limited (Figure 1). Commercially available 50 mg of Riluzole tablets (Rilutek®) were purchased from Pharmaceuticals Limited, Mumbai, India. HPLC grade acetonitrile, analytical reagent grade ammonium acetate and triethylamine were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurities used in this study were of greater than 99.0% purity.

## Equipment

The LC system, used for method development, forced degradation studies and method validation was Waters 2695 binary pump plus auto sampler and a 2996 photo diode array detector. The output signal was monitored and processed using Empower software on Pentium computer (Digital equipment Co). Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Mack Pharmatech, Hyderabad, India).

## Chromatographic conditions

The chromatographic column used was Zorbax SB C18 (150 × 4.6) mm with 5µm particles. The mobile phase consists of buffer and acetonitrile in the ratio (60:40, v/v). Buffer consists of 10 mM ammonium acetate, pH adjusted to 8.5 using triethylamine. The column temperature was maintained at 35°C and the de-

tection was monitored at a wavelength of 241 nm. The injection volume was 20  $\mu$ L. Mobile phase was used as diluent.

### Preparation of solutions

#### Preparation of standard solutions

A stock solution of Riluzole (2.0 mg mL<sup>-1</sup>) was prepared by dissolving appropriate amount in the diluent. Working solutions were prepared from above stock solution for related substances determination and assay determination, respectively. A stock solution of impurities (mixture of imp-1, imp-2 and imp-3) at a concentration of 1.0 mg mL<sup>-1</sup> was also prepared in diluent.

#### Preparation of sample solutions

Rilutek® tablets contain 50 mg of Riluzole. The inactive ingredients present in Rilutek® are anhydrous dibasic calcium phosphate, microcrystalline cellulose, anhydrous colloidal silica, magnesium stearate, croscarmellose sodium. Twenty Rilutek® tablets (50 mg) were weighed and the average weight was calculated. The tablets were powdered in a mortar and a sample of the powder equivalent to 100 mg of the active pharmaceutical ingredient (Riluzole) was transferred to 100 mL volumetric flask. Approximately 75 mL diluent was added and the flask was placed on rotatory shaker for 10 min and sonicated for 10 min to dissolve the material completely. The solution was then diluted to 100 mL and centrifuged at 3,000 rpm for 10 min. The supernatant was collected and filtered through a 0.45  $\mu$ m pore size Nylon 66-membrane filter. The filtrate was used as sample solution.

#### Analytical method validation

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and system suitability.

#### Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the developed LC method for

Riluzole was determined in the presence of its impurities namely imp-1, imp-2, imp-3, and degradation products. Forced degradation studies were also performed on Riluzole to provide an indication of the stability indicating property and specificity of the proposed method. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60°C), acid hydrolysis (1N HCl), base hydrolysis (1N NaOH), water hydrolysis and oxidation (6% H<sub>2</sub>O<sub>2</sub>). For heat and light studies, study period was 10 days where as for acid, base, peroxide and water hydrolysis the test period was 48 h. Peak purity of stressed samples of Riluzole was checked by using 2996 Photo diode array detector of Waters (PDA).

#### Analytical method validation

##### Precision

The precision of the related substance method was checked by injecting six individual preparations of (1000  $\mu$ g mL<sup>-1</sup>) Riluzole spiked with 0.15% each imp-1, imp-2 and imp-3. The %RSD of area for each imp-1, imp-2 and imp-3 was calculated. Precision study was also determined by performing the same procedures on a different day (interday precision).

The intermediate precision (ruggedness) of the method was also evaluated using different analyst, different column and different instrument in the same laboratory.

Assay method precision was evaluated by carrying out six independent assays of test sample of Riluzole against qualified reference standard. The %RSD of six assay values obtained was calculated. The intermediate precision of the assay method was evaluated by different analyst and by using different instrument from the same laboratory.

##### Sensitivity

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for imp-1, imp-2 and imp-3 estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of imp-1, imp-2 and imp-3, calculated the %RSD for the areas of each impurity.

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### Linearity

Linearity test solutions for assay method were prepared from stock solution at five concentration levels from 50 to 200% of assay analyte concentration (50, 75, 100, 150, and 200  $\mu\text{g mL}^{-1}$ ).

Linearity test solutions for related substance method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels. From LOQ to 200% of the permitted maximum level of the impurity (i.e. LOQ, 0.075, 0.1125, 0.15, 0.1875, 0.225 and 0.30% was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses.

### Accuracy

The accuracy of the assay method was evaluated in triplicate at five concentration levels, i.e. 50, 75, 100, 150 and 200  $\mu\text{g mL}^{-1}$  in bulk drugs and pharmaceutical dosage forms. For each concentration, three sets were prepared and injected in triplicate. The percentages of recoveries were calculated.

The bulk sample shows the presence of imp-3 at a level of 0.07% and it shows a total of 0.07% of impurities (limit: not more than 0.15% for single unknown impurity, for total impurities the limit is 0.50%). The study was carried out in triplicate at LOQ, 0.075, 0.15, and 0.225 of the analyte concentration ( $1000\mu\text{g mL}^{-1}$ ). The percentage of recoveries for imp-1, imp-2 and imp-3 were calculated.

### Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution ( $R_s$ ) between Riluzole, imp-1, imp-2 and imp-3 were evaluated. The flow rate of the mobile phase was  $1.2\text{ mL min}^{-1}$ . To study the effect of flow rate on the developed method, 0.2 units of flow changed (i.e. 1.0 and  $1.4\text{ mL min}^{-1}$ ). The effect of column temperature on the developed method was studied at  $30^\circ\text{C}$  and  $40^\circ\text{C}$  instead of  $35^\circ\text{C}$  and use of a column from a different batch. The effect of pH on resolution of impurities was studied by varying  $\pm 0.1$  pH units (i.e. buffer pH altered from 8.5 to 8.4 and 8.6). In the all above varied conditions, the components of the

mobile phase were held constant.

### Solution stability and mobile phase stability

The solution stability of Riluzole in the assay method was carried out by leaving the test solutions of samples in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed 6 h interval up to the study period against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions 6 h interval up to 48 h. Mobile phase prepared was kept constant during the study period of mobile phase stability. The %RSD of assay of Riluzole was calculated for the study period during mobile phase and solution stability experiments.

The solution stability of Riluzole and its related impurities were carried out by leaving both spiked sample and unspiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content of imp-1, imp-2 and imp-3 were determined every 6 h interval up to the study period.

Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Content of imp-1, imp-2 and imp-3 was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

## RESULTS AND DISCUSSION

### Method development and optimization

The objective of the study was to develop a chromatographic method for a separation of closely eluting impurities namely imp-1, Riluzole and imp-2 and symmetry of Riluzole peak. Riluzole sample spiked (0.15%) with all the impurities was used during method development. To develop a rugged and suitable LC method different mobile phases and stationary phases were investigated. The proportion of the mobile phase components was optimized to reduce retention times and enable good resolution of Riluzole from the spiked solution. A detection wavelength of 241 nm was selected after scanning the standard solution over the range 190-370 nm by use of the PDA. Detection at 241 nm resulted in good response and good linearity. Preliminary trial was carried out with Inertsil C18, 25 cm length,

4.6 mm ID and 5 $\mu$ m particle size with the mobile phase consisting of ammonium acetate buffer (5 mM) with pH 6.5 and acetonitrile (50:50, v/v), delivered in a flow rate of 1.0 mL min<sup>-1</sup> resulted the poor peak shape and the imp-2 is merging with Riluzole was observed.

The Zorbax SB C18 column was used because of its advantages of high retention, high resolving capacity, better reproducibility, low-back pressure, and low tailing. Introduction of Zorbax SB C18 has given satisfactory results in terms of symmetry  $\sim$ 1.5 and found marginal improvement in the resolution. To further improve the symmetry of Riluzole peak, the temperature was increased to 35°C and observed the improvement in symmetry ( $\sim$ 1.1).

Owing to the basic nature the pH of the buffer was adjusted to 8.5 with triethylamine and observed that the symmetry of the peak was improved and the resolution between the impurities also improved but the retention time of Riluzole peak was about 20 min. To minimize the retention time of Riluzole the column length was changed to 15 cm length, 4.6 mm ID and 5 $\mu$ m particle size and the flow rate was optimized to 1.2 mL min<sup>-1</sup> and satisfactory results were obtained.

In the optimized conditions Riluzole, imp-1, imp-2 and imp-3 were well separated with a resolution ( $R_s$ ) of greater than 2.0 and the typical retention times of imp-1, Riluzole, imp-2, and imp-3 were about 3.9, 6.2, 7.0 and 11.2 min respectively. The system suitability results were given in (TABLE 1) and the developed LC method was found to be specific for Riluzole and its impurities namely imp-1, imp-2 and imp-3 (TABLE 2).

## Results of forced degradation studies

### Degradation in acidic solution

The drug was stable to the effect of acid hydrolysis. When the drug powder was exposed to 1 N HCl at 100°C for 48 h, no degradation was observed.

### Degradation in basic solution

The drug was stable to the effect of base hydrolysis. When the drug powder was exposed to 1 N NaOH at 100°C for 48 h, no degradation was observed.

### Oxidative conditions

Riluzole has shown significant sensitivity towards

TABLE 1 : System suitability report

Compd.	USP Resolution ( $R_s$ )	USP tailing factor (T)	No of theoretical plates USP tangent method (N)
Imp-1	-	1.0	9298
Riluzole	11.6	1.1	10957
Imp-2	3.2	0.9	12054
Imp-3	12.4	1.0	12686

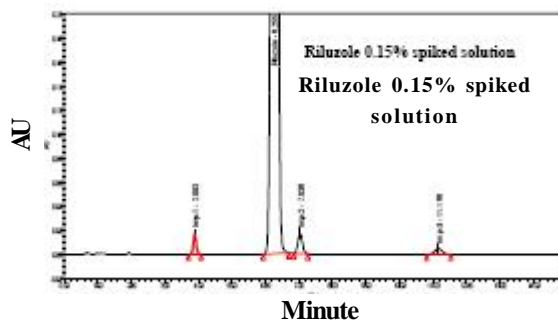


Figure 2 : Typical chromatogram of Riluzole 0.15% spiked solution

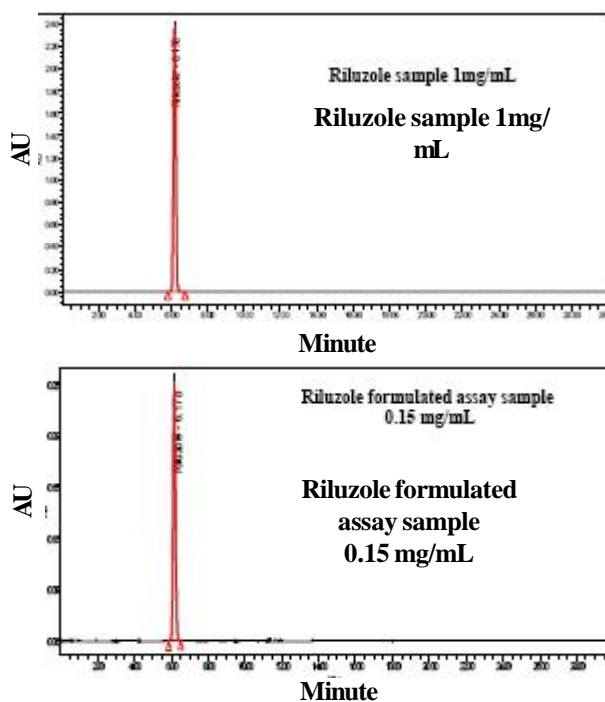


Figure 3 : Riluzole formulated assay sample 0.1 mg/mL

the treatment of 6% hydrogen peroxide at 100°C for 4 h and the drug gradually undergone oxidative degradation and it was degraded up to  $\sim$ 10% (Figure 4).

### Degradation in neutral (Water) solution

The drug was stable to the effect of water hydrolysis. No degradation was observed after 48 h at room temperature.

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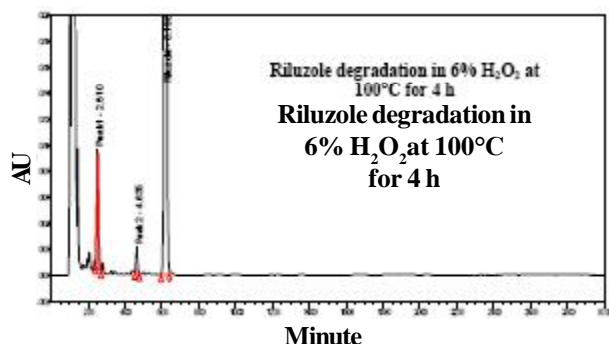
Figure 4 : Riluzole degradation in 6% $H_2O_2$ 

TABLE 2 : Summary of forced degradation results

Stress condition	Time	Mass balance	
		%Assay of active substance	(%Assay + %Impurities + %Degradation products)
Acid hydrolysis (1N HCl)	48 h	100	99.93
Base hydrolysis (0.1N NaOH)	48 h	100	99.93
Oxidation (6% $H_2O_2$ )	4 h	90	99.93
Water degradation	48 h	100	99.93
Light (photolytic degradation)	10 days	100	99.93
Thermal (100°C)	10days	100	99.93

TABLE 3 : Results of intermediate precision

S. no	Parameter	Variation	%RSD for assay	%RSD For related substances	Resolution ( $R_s$ ) between Riluzole and imp-2
1	Different system	(a) Waters 2695 Alliance system	0.1	< 2.0	3.2
		(b) Agilent 1100 series VWD system	0.2	< 2.0	3.1
2	Different column	(a) B.No:11125	0.2	< 2.0	3.2
		(b) B.No:11041	0.3	< 2.0	3.2
3	Different analyst	(a) Analyst-1	0.1	< 2.0	3.2
		(b) Analyst-2	0.2	< 2.0	3.1

### Photolytic conditions

The drug was stable to the effect of photolysis. When the drug powder was exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200-watt hours/square meter (w/mhr) (in photo stability chamber), no degradation was observed.

### Thermal degradation

The drug was stable to the effect of temperature. When the drug powder was exposed to dry heat at 100°C for 10 days, no degradation was observed.

From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the Riluzole peak was homogeneous and pure in all the analyzed stress samples. No degradants were observed after 30 min in the extended runtime of 60 min of all the Riluzole samples. The assay of Riluzole is unaffected in the presence of imp-1, imp-2 and imp-3 and its degradation products confirm the stability indicating power of the developed method.

### Method validation

#### Precision

The %RSD of assay of Riluzole during assay method precision study was 0.2% and the %RSD of area of imp-1, imp-2 and imp-3 in related substance method precision study were within 2.0%. Confirming the good precision of the developed analytical method (Figure 2).

The %RSD of assay results obtained in intermediate precision study was within 0.3% and the %RSD for imp-1, imp-2 and imp-3 were well within 2.0%, confirming the ruggedness of the method (TABLE 3).

#### Sensitivity

The limit of detection of imp-1, imp-2 and imp-3 were 0.0003, 0.0004 and 0.001% (of analyte concentration, i.e. 1000 $\mu\text{g mL}^{-1}$ ) respectively for 10 $\mu\text{L}$  injection volume. The limit of quantitation of imp-1, imp-2 and imp-3 were 0.001, 0.001 and 0.003% (of analyte concentration, i.e. 1000 $\mu\text{g mL}^{-1}$ ) respectively for 10 $\mu\text{L}$  injection volume. The precision at LOQ concentration for imp-1, imp-2 and imp-3 were below 2.0%.

#### Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 50-200 $\mu\text{g mL}^{-1}$  and the correlation coefficient obtained was greater than 0.999. The result shows an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.225% for imp-1, imp-2 and imp-3. The cor-

**TABLE 4 : Results of accuracy study for drug substance**

Added ( $\mu\text{g}$ )(n= 3)	Recovered( $\mu\text{g}$ )	%Recovery	%R.S.D
250	249.5	99.8	0.7
375	379.1	101.1	0.8
500	491.5	98.3	0.2
750	745.5	99.4	0.3
1000	991.0	99.1	0.2

n =3, Number of determinations

**TABLE 5 : Results of accuracy study for drug product**

Added ( $\mu\text{g}$ ) (n= 3)	Recovered ( $\mu\text{g}$ )	%Recovery	%R.S.D
250	252.0	100.8	0.4
375	385.5	102.8	0.2
500	515.5	103.1	0.5
750	757.5	101.0	0.6
1000	1002.0	100.2	0.2

n =3, Number of determinations

**TABLE 6 : Results of accuracy study for impurities**

No. of accuracy level (n= 3)	%	%	%
	Imp-1	Imp-2	Imp-3
Accuracy at LOQ	101.5	100.1	99.8
Accuracy at 50% level	100.8	101.1	101.0
Accuracy at 100% level	100.3	100.8	100.1
Accuracy at 150% level	101.7	99.7	101.5

n =3, Number of determinations

**TABLE 7 : Results of robustness study**

S. no.	Parameter	Variation	Resolution ( $R_s$ ) between Riluzole and imp-2
1	Temperature ( $\pm 5^\circ\text{C}$ of set temperature)	(a) At $30^\circ\text{C}$	3.2
		(b) At $40^\circ\text{C}$	3.3
2	Flow rate ( $\pm 20\%$ of the set flow)	(a) At $0.8\text{mLmin}^{-1}$	3.3
		(b) At $1.2\text{mLmin}^{-1}$	3.1
3	pH ( $\pm 0.1$ unit of set pH)	(a) At 8.4	3.1
		(b) At 8.6	3.3

**TABLE 8 : Batch analysis for Riluzole drug substance**

Batch no:	Imp-1 (%)	Imp-2 (%)	Imp-3 (%)	Assay by HPLC (%)
0304	ND	ND	0.07	99.95
0308	ND	ND	0.07	99.96
0315	ND	ND	0.07	99.95

Where ND = Not Detected

**TABLE 9 : Batch analysis for Riluzole drug product**

Batch no.	Assay by HPLC (%)
RXS-789	103.5
RXS-801	101.8
RXS-855	101.9

relation coefficient obtained was greater than 0.999 for all three impurities. The result shows an excellent correlation existed between the peak area and concentra-

tion of imp-1, imp-2 and imp-3.

## Accuracy

The percentage recovery of Riluzole in bulk drug samples ranged from 98.3- 101.1% (TABLE 4) and in pharmaceutical dosage forms ranged from 101.0-103.1% (TABLE 5). The percentage recovery of imp-1, imp-2 and imp-3 in bulk drug samples ranged from 99.7-101.7% (TABLE 6). HPLC chromatograms of spiked sample with all three impurities in Riluzole bulk drug sample are shown in figure 2.

## Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, pH and column temperature) revealed that the resolution between Riluzole and closely eluting impurity, namely imp-2 was always greater than 2.0, illustrating the robustness of the method (TABLE 7).

## Solution stability and mobile phase stability

The %RSD of assay of Riluzole during solution stability and mobile phase stability experiments was within 1.0. No significant changes were observed in the content of imp-1, imp-2 and imp-3 during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related substance determination were stable up to the study period of 48 h.

## Assay analysis

Analysis was performed for different batches of Riluzole in both bulk drug samples (n=3) and dosage forms (n=3). Results were given in TABLES 8 and 9.

## CONCLUSION

The RP-LC method developed for quantitative and related substance determination of Riluzole in both bulk drugs and pharmaceutical dosage forms are precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Riluzole samples.

## ACKNOWLEDGMENTS

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## REFERENCES

- [1] J.H.Song, C.S.Huang, K.Nagata, J.Z. Yeh, T. Narahashi; *J.Pharmacol.Exp.Ther.*, **282**, 707-14 (1997).
- [2] V.Coric, S.Taskiran, C.Pittenger, S.Wasylink, D.H. Mathalon, G.Valentine, J.Saksa, Y.T.Wu, R. Gueorguieva, G.Sanacora, R.T.Malison, J.H.Krystal; *Biol.Psychiatry*, (2005).
- [3] S.J.Mathew, J.M.Amiel, J.D.Coplan, H.A.Fitterling, H.A.Sackeim, J.M.Gorman; *Am.J.Psychiatry*, **162**, 2379-2381 (2005).
- [4] Elena Fumagalli, Marcella Funicello, Thomas Rauen, Marco Gobbi, Tiziana Mennini; *European Journal of Pharmacology*, **578**, 171-176 (2008).
- [5] H.J.Van Kan, G.J.Groeneveld, S.Kalmijn, M. Spieksma, L.H.Van den Berg, H.J.Guchelaar; *Association Br.J.Clin.Pharmacol.*, **59**, 310-3 (2005).
- [6] C.A.Zarate (Jr.), J.L.Payne, J.Quiroz, J.Sporn, K.K. Denicoff, D.Luckenbaugh, D.S.Charney, H.K. Manji; *Am.J.Psychiatry*, **161**, 171-4 (2004).
- [7] Milena Colovic, Eleonora Zennaro, Silvio Caccia; *J.Pharm.B*, **803**, 305-309 (2004).
- [8] Xue-Ding Wang, Eli Chan, Xiao Chen, Xiao-Xing Liao, Cheng Tang, Zhi-Wei Zhou, Min Huang, Shu-Feng Zhou; *J.Pharm.Biomed.Anal.*, **44**, 224-230 (2007).
- [9] ICH guidelines on Validation of Analytical procedures, Text and Methodology Q2 (R<sub>1</sub>), FDA, Published in the Federal Register, **60**, 1260 (1995).
- [10] USP; United States pharmacopoeia, 31<sup>th</sup> Edn., United States Pharmacopoeial Convention, Rockville, (2008).
- [11] M.Bakshi, S.Singh; *J.Pharm.Biomed.Anal.*, **28**, 1011-1040 (2002).