ORIGINAL ARTICLE

Development and Validation of Reverse-phase High-performance Liquid Chromatography Method for Simultaneous Estimation of Riluzole and Levodopa in Tablet Dosage Form

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Abstract

Objective: The objective of this study was to develop a new and simple reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of riluzole (RZ) and levodopa (LD) in the marketed tablet dosage form. **Materials and Methods:** The chromatographic separation was achieved on a RP hypercil C_{18} column (250 × 4.6 mm i.d., 5 µm) using a mobile phase consisting of methanol and water (75:25 v/v) at a flow rate of 1.0 ml/min with the ultraviolet detection wavelength of 273 nm at ambient temperature. The developed method was validated as per the International Council on Harmonization guideline for linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), and specificity. **Results:** Results of validation studies were found satisfactory with % relative standard deviation values of <2% indicating good specificity, validity, and reliability of the method. The method showed good linearity over the concentration range of 20–80 µg/ml with correlation coefficient (r^2) values of 0.990and 0.999 for RZ and LD, respectively. The mean percentage recoveries were between 99.46–99.80% and 100.15–101.24% for RZ and LD, respectively. The LOD and LOQ values were found to be 0.036and 0.012 µg/ml, and 0.110and 0.036 µg/ml for RZ and LD, respectively. The assay of RZ and LD in the marketed tablet formulation was found to be 99.67and 98.95%, respectively. **Conclusion:** The RP-HPLC method is reported to be simple, specific, accurate, and precise. The proposed method can be successfully applied for the routine analysis of RZ and LD in the bulk drugs as well as in combined pharmaceutical dosage forms.

Key words: International Council on Harmonization guidelines, Levodopa, Method validation, Reverse-phase high performance liquid chromatography, Riluzole, Tablet dosage form

INTRODUCTION

Riluzole (RZ), 2-amino-6-(trifluoromethoxy) benzothiazole, Figure 1A is a neuroprotective drug used in the treatment for neurologic disorders such as amyotrophic lateral sclerosis (ALS). It also has sedative and anticonvulsant properties.[1,2] RZ blocks glutamatergic neurotransmission in the central nervous system (CNS) by

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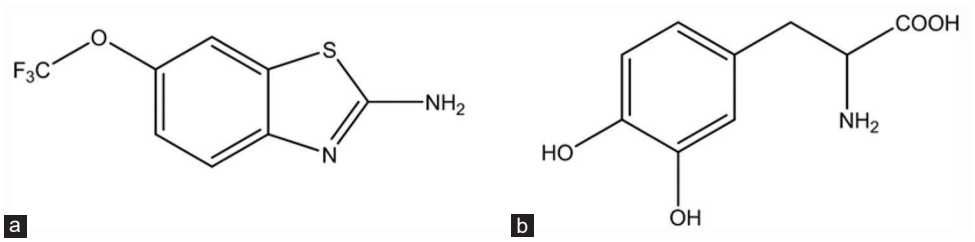

Figure 1: Chemical structures of (a) riluzole and (b) levodopa

non-competitive blockade of glutamic acid at N-methyl-D-aspartate receptors.[3,4] Recent studies indicate that RZ can be clinically used in the treatment of depression and anxiety disorders.[5,6] RZ occurs as white to slightly yellow and odor less solid which is very soluble in methanol, freely soluble in dichloromethane, sparingly soluble in 0.1N HCl, and very slightly soluble in water.[7] On the other hand, levodopa (LD), an aromatic amino acid (LD, 2-amino-3-(3,4-dihydroxyphenyl) propanoic acid, Figure 1B), is an antiparkinsonian drug used in the management for Parkinson's disease.[8,9] In the CNS of patients with Parkinson's disease, LD is converted into dopamine by dopa decarboxylase enzyme and can treat Parkinson's disease.[10] LD is a white and crystalline solid which is slightly soluble in water, soluble in mineral acids and alkali carbonates.[11]

Several analytical methods have been reported for the individual determination of RZ and LD in pharmaceutical dosage forms using ultraviolet (UV) spectrophotometry, RP-HPLC, etc.[5-7,9,10] Literature survey reveals that there are no analytical methods reported so far for the simultaneous estimation of RZ and LD in combined pharmaceutical dosage forms. In this paper, a simple, accurate, and precise RP-HPLC method was developed and validated for the simultaneous estimation of RZ and LD in the marketed tablet dosage form.

MATERIALS AND METHODS

Chemicals

Pure drugs of RZ RS and LD RS were obtained from the Glenmark Pharmaceuticals Ltd., Mumbai, India. RZ and LD tablets were procured form the local medical store. HPLC grade methanol and water were obtained from Merck Pvt. Ltd., Mumbai, India.

HPLC instrument and chromatographic conditions

Chromatographic separation was achieved on a Water 2690 series Liquid Chromatographic system equipped with a pump, autosampler, and a photodiode array detector . The chromatographic column was a Thermo Hypersil-ODS C_{18} column (250 mm × 4.6 mm i.d., 5 µm). The mobile phase consisting of methanol and water was in a ratio of 75:25 v/v. Isocratic elution was carried out at ambient temperature. The flow rate was 1.0 ml/min and the injection volume was 20 µl. The UV detection wavelength was set at 273 nm.

Standard solutions

Accurately 10 mg of RZ RS and 10 mg of LD RS were weighed separately into two 10 ml of volumetric flasks, dissolved, and diluted with methanol, followed by sonicated for 20 min to obtain a concentration of 1000 µg/ml. A working stock solution equivalent to 50 µg/ml was prepared, sonicated, and filtered through 0.45 µ membrane filter.

Sample solutions

Twenty tablets of each marketed tablet formulation of RZ and LD were accurately weighed and powdered. The powder equivalent to 5 mg of the active ingredient was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml of methanol and sonicated for 15 min. Finally, the solution was made up to the volume with methanol to obtain a concentration of 50 µg/ml and filtered through 0.45 µ membrane filter.

RESULTS AND DISCUSSION

Method development

Several trial runs were performed using C_8 and C_{18} reversed-phase columns, various mobile phase compositions and different flow rates for the separation of RZ and LD with good chromatographic parameters such as resolution, theoretical plates, and tailing factor. A C_{18} column (250 mm × 4.6 mm, i.e., 5 µm) used as the stationary phase and a mobile phase consisting of methanol/water (75:25 v/v) at a flow rate of 1.0 ml/min and a UV detection wavelength of 273 nm for a run time of 8 min afforded the best separation with well-resolved and sharp peaks for both the drugs. The retention time (Rt) for RZ and LD was 2.948 min and 4.189 min, respectively. The chromatogram of the optimized method is presented in Figure 2.

Method validation

The developed RP-HPLC method was validated in terms of the following parameters according to the International Conference on Harmonization (ICH) guidelines. Linearity,

accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), specificity, and system suitability studies were carried out.[12-19]

Linearity

The linearity was evaluated by analyzing six (n = 6) standard solutions of RZ and LD for a concentration range of 20–80 µg/ml. The calibration curve was constructed by plotting a graph between peak area and concentration. The straight-line equation was determined. The calibration plot was found linear in the range between 20 and 80 µg/ml for both RZ and LD. The regression equations were obtained as follows: y =17543x + 1829.1 (r^2=0.990) for RZ [Figure 3A], and y = 20802x+ 3069.4 (r^2=0.999) for LD [Figure 3B], where y = peak area, x = concentration of solution; and r = the square of determined correlation coefficient. The developed RP-HPLC method was linear over the specified range.

Accuracy

In accuracy study, a known amount of the standard drug was added to the sample solution to obtain three different concentrations. This study was performed at three spiked levels, that is, 50, 100, and 150% for triplicate observations (n = 3). The average percent recoveries were in the range between 99.0 and 110.0% for both RZ and LD. Results of recovery studies were found to be satisfactory. The % recovery data are summarized in Table 1.

Precision

Repeatability (intra-day precision) was determined by injecting six replicate (n = 6) solutions of the standard concentration (4 µg/ml, 100%) for each drug in the same day. Similarly, reproducibility (inter-day precision) was performed by analyzing six samples (n = 6) of standard concentration (40 µg/ml, 100%) for each drug in the same laboratory on

Table 1: Accuracy (% recovery) data for RZ and LD

Drug	Spiked level (µg/ml)	Concentration (µg/ml)		
		Amount added	Amount recovered	Mean %Recovery*
RZ	50	20.00	19.88	99.460
	100	40.00	39.89	99.747
	150	60.00	59.87	99.800
LD	50	20.00	20.02	100.155
	100	40.00	40.38	100.958
	150	60.00	60.74	101.240

RZ: Riluzole; LD: Levodopa; *mean of three replicate determinations (n=3)

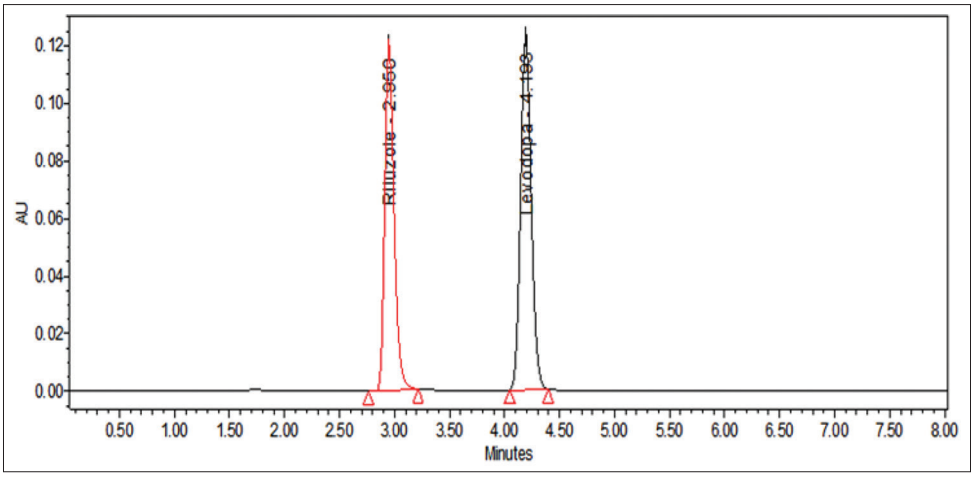

Figure 2: Chromatogram of the optimized method

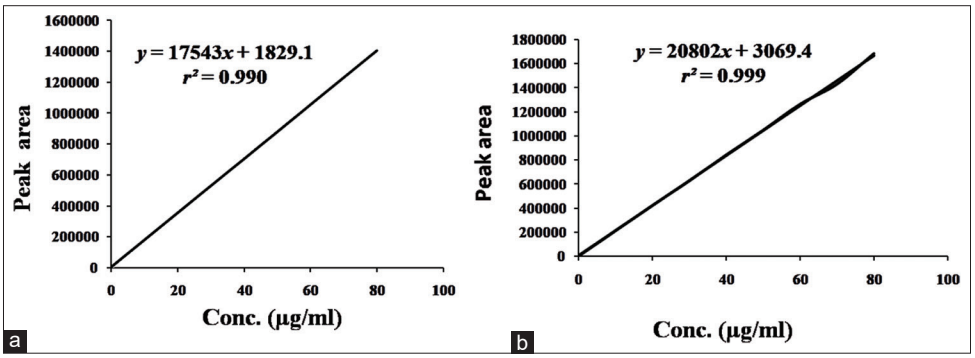

Figure 3: Calibration curve of (a) riluzole and (b) levodopa

different day under similar experimental conditions. Relative standard deviation (RSD) values were calculated. Results of precision studies were found to be satisfactory. The %RSD values were <2% indicating good repeatability as well as reproducibility of the RP-HPLC method. Results of precision studies are presented in Table 2.

Ruggedness

Ruggedness was determined by analyzing six samples (n = 6) of the standard concentration (4 µg/ml) by two different analysts in the same laboratory under similar experimental conditions. Results of ruggedness studies are displayed in Table 3. The %RSD values were <2%, which proved good ruggedness of the developed method.

Robustness

The robustness was studied by injecting six replicates (n = 6) of standard solution (4 µg/ml) by introducing small changes in the chromatographic condition, that is, flow rate. Results are depicted in Table 4. The developed method was practically robust. The % RSD values determined under robustness conditions were <2.0%.

LOD and LOQ

The LOD corresponding to a signal-to-noise ratio of 3 was found to be 0.036 µg/ml and 0.012 µg/ml for RZ and LD, respectively. The LOQ corresponding to a signal-to-noise ratio of 10 was 0.110 µg/ml and 0.036 µg/ml for RZ and LD, respectively. From the LOD and LOQ values, it was clear that the developed method was sensitive for the precise simultaneous determination of RZ and LD.

Specificity

The specificity of the method was demonstrated by the separation of the analytes of interest from other potential components such as excipients, impurities, and related active principles.[20,21] A volume of 20 µl of sample solution (4 µg/ml) was injected and the chromatogram was recorded. No peaks were found in the chromatogram other than the peaks due to RZ and LD with the Rt of 2.948 min and 4.189 min Results showed that the method was free from interference due to excipients, impurities, or other related components [Figure 4]. The proposed method is, therefore, claimed to be to be specific for the quantitative simultaneous determination of RZ and LD in pharmaceutical formulations.

System suitability

System suitability was determined by injecting six replicate injections of the standard solution (4 µg/ml) of RZ and LD. Results of system suitability parameters (resolution, theoretical plates, tailing factor, etc.)[22] were found within the limit with %RSD values of <2%). The summary of validation

Table 2: Results of precision studies

Precision*	Repeatability (Intra-day)*	Reproducibility (Inter-day)*
RZ		
%Assay	99.700±0.030	99.707±0.011
%RSD	0.030	0.011
LD		
%Assay	100.956±0.055	100.957±0.004
%RSD	0.055	0.004

RZ: Riluzole, LD: Levodopa, RSD: Relative standard deviation. *mean±SD of six replicate observations (n=6)

Table 3: Results of Ruggedness

Ruggedness*	Analyst 1	Analyst 2
RZ		
%Assay	99.700±0.030	99.709±0.007
%RSD	0.030	0.007
LD		
%Assay	100.956±0.005	100.955±0.009
%RSD	0.005	0.008

RZ: Riluzole, LD: Levodopa, RSD: Relative standard deviation. *mean±SD of six replicate observations (n=6)

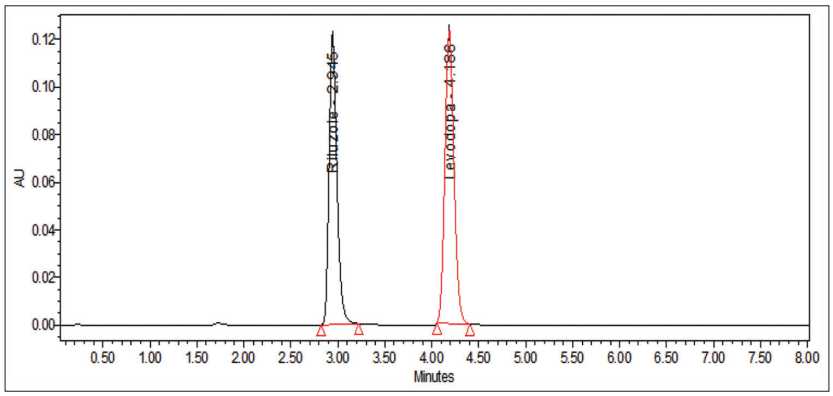

Figure 4: Chromatogram showing non-interference of analyte peaks

Table 4: Results of robustness

Robustness*	Modification in flow rate		
	0.8 ml/min	1.0 ml/min	1.2 ml/min
RZ			
Peak area	882627 ±0.002	701563 ±0.003	57797 5±0.007
%RSD	0.002	0.003	0.0.007
LD			
Peak area	105163 ±0.020	841139 ±0.004	682026 ±0.006
%RSD	0.020	0.004	0.006

RZ: Riluzole, LD: Levodopa, RSD: Relative standard deviation. *mean±SD of six replicate observations (n=6)

parameters are represented in Table 5. In system suitability test, the method produced excellent separation of the analyte peaks with good resolution between the two analytes [Figure 5]. Moreover, higher percentage of recovery and non-interference of the formulation excipients in Rt of the analytes exhibited the selectivity of the method for the simultaneous estimation of both the drugs in the combined formulation.

Assay (estimation of RZ and LD in marketed formulation)

The assay of RZ and LD in the marketed tablet formulation was found to be 99.67% and 98.95%, respectively [Table 6].

Table 5: Summary of validation and system suitability parameters

Parameters	Acceptance criteria	Result	
		RZ	LD
Linearity			
Range (µg/ml)	-	20–80	20–80
Slope	-	17.543	120.802
Intercept	-	1829.1	3069.4
Coefficient of correlation (r^2)	NLT 0.997	0.990	0.999
Accuracy or %Recovery			
50	99–110%	99.460	100.155
100		99.747	100.958
150		99.800	101.240
Precision			
Repeatability (%RSD)	NMT 2%	0.030	0.011
Reproducibility (%RSD)		0.055	0.004
Ruggedness (%RSD)		0.030	0.005
Robustness (%RSD)		0.003	0.004
LOD (µg/ml)	-	0.036	0.012
LOQ (µg/ml)	-	0.110	0.036
System suitability			
Rt, min.	-	2.948	4.189
Resolution	<2	7.30415	7.30415
Peak area	-	701773	831092
%RSD	<2	0.02786	0.00906
Theoretical plates	<3000	6068	8958
Tailing factor	<2	1.243	1.129

RZ: Riluzole; LD: Levodopa; RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantitation

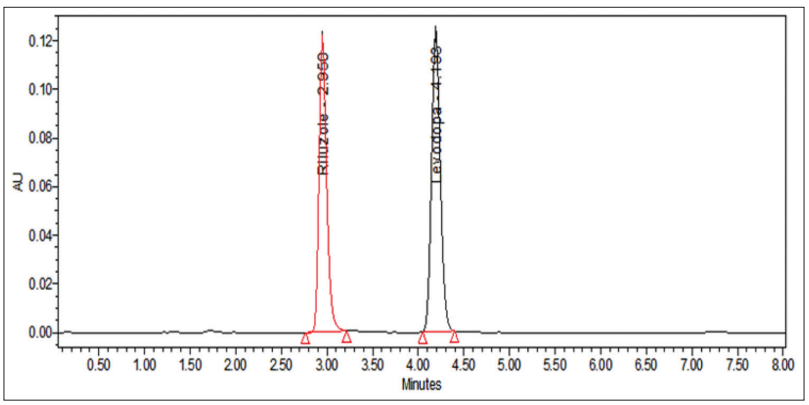

Figure 5: Chromatogram of system suitability test

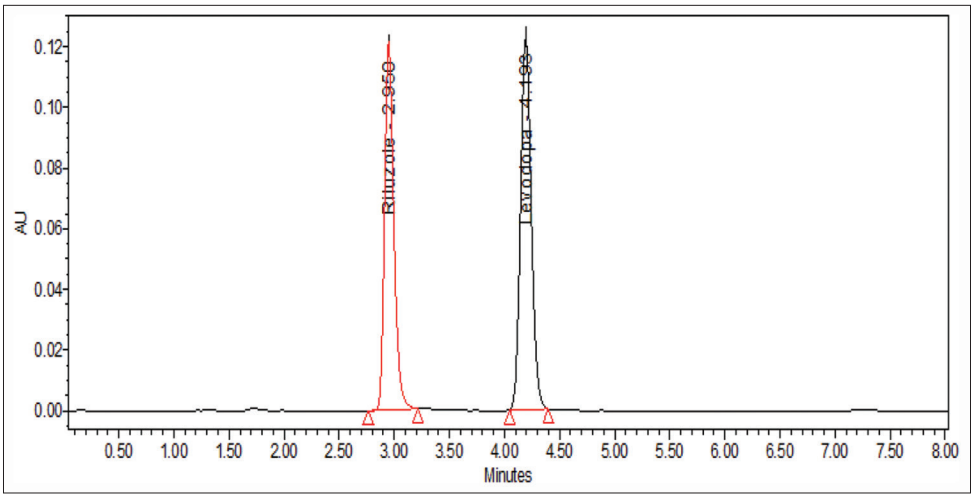

Figure 6: Assay chromatogram for marketed formulation

Table 6: Assay of RZ and LD in marketed tablet dosage form

Peak area*	Amount recovered (µg/ml)	% Estimated
RZ		
701773±0.030	39.905	99.67
LD		
831092±0.005	40.384	98.95

RZ: Riluzole; LD: Levodopa; *mean±SD of three replicate observations (n=3)

The chromatogram of RZ and LD in the combined marketed formulation is presented in Figure 6. The mean percentage (n = 3) estimated was in good agreement with the label claimed. Results of assay imply that the proposed method can be applied for the simultaneous determination of RZ and LD in the combined pharmaceutical dosage forms.

CONCLUSION

In this study, a new RP-HPLC method was successfully developed and validated for the simultaneous estimation of RZ and LD in the combined marketed tablet dosage form. The proposed method is claimed to be simple, accurate, and precise. The developed method is free from interferences due to excipients, impurities, or related substances. The developed method is also reported to be specific, valid, and reliable. This method can, therefore, be used for the routine analysis of RZ and LD in bulk drugs as well as in pharmaceutical dosage forms.

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