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Research Article

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Development and Validation of Stability-indicating HPTLC Method for Determination of Rizatriptan as Bulk Drug and in Tablet Dosage Form

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ABSTRACT

A new simple, accurate, precise and selective stability- indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for estimation of Rizatriptan in tablet dosage form. The mobile phase selected was Benzene: Methanol (8: 2, v/v) with UV detection at 227 nm. The retention factor for Rizatriptan was found to be 0.50 ± 0.006. The method was validated with respect to linearity, accuracy, precision and robustness as per ICH guidelines. The drug was subjected to stress condition of hydrolysis (acid, base), oxidation and thermal degradation. Results found to be linear in the concentration range of 500-2500 ng band^{-1}. The method has been successfully applied for the analysis of drug in pharmaceutical formulation. The % assay (Mean±S.D.) was found to be 99.78±1.38. The developed method can be used for quantitative determination and checking the stability of Rizatriptan in bulk drug and pharmaceutical dosage form.

Keywords: Rizatriptan, HPTLC, Forced degradation, Validation

INTRODUCTION

Rizatriptan (RIZT), chemically, N,N- dimethyl amino-2-[5-(1H-1,2,4-triazol-1-yl methyl) -1H- indol -3-yl] ethanamine is a selective serotonin receptor agonist of the 1B and 1D sub types andused in the acute treatment of migraine attacks [1]. Literature survey revealed that UV spectrophotometric [2, 3], HPLC [4, 5] and LC-MS [6, 7] methods have been reported for the determination of RIZT in pharmaceutical dosage form and in human plasma. Optimization and validation of HPLC assay for RIZT and its impurities in tablets by using chemometric approach has also been reported [8].

To best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of RIZT in tablet dosage form. The present work describes simple, precise, accurate and selective HPTLC method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per International Conference on Harmonization Guidelines [9, 10].

EXPERIMENTAL SECTION

Chemicals and reagents

Pharmaceutical grade working standard RIZT was kindly supplied by Getz Pharma Research Pvt. Ltd. (Thane, India). The pharmaceutical dosage form used in this study was Rizact tablets (CiplaLtd., India) labeled to contain 10

mg of RIZT was procured from the local market. Benzene, Methanol (HPLC grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on precoated silica gel aluminium plate 60 F_{254} (10×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using benzene: methanol (8:2, v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 227 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solution

Working standard solution was prepared by dissolving 10 mg of drug in 10 mL of methanol to get concentration of 1 mg mL^{-1} from which 5 mL was further diluted to 10 mL with methanol to get solution of 500 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 227 nm. So, 227 nm was selected as the wavelength for detection.

Tablet formulation analysis

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of Rizatriptan was weighed and transferred to a 10 mL volumetric flask containing approximately 5 mL of methanol and the content was sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 1000 ng $band^{-1}$. Fivemililitre volume of above solution was diluted with methanol to obtain final concentration of 500 ng $band^{-1}$. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 1000 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 227 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid/base hydrolytic, oxidative, thermolytic stress conditions.

Acid induced degradation

1 mL working standard solution of RIZT (1000ng μL^{-1}) was mixed with 1 mL of 0.1 N methanolicHCl and 8 mL of methanol. Solution was kept at room temperature for 15 min. 15 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. 24.65 % of degradation was observed with two additional degradation products. The representative densitogram obtained after acid treatment is shown in Figure 1.

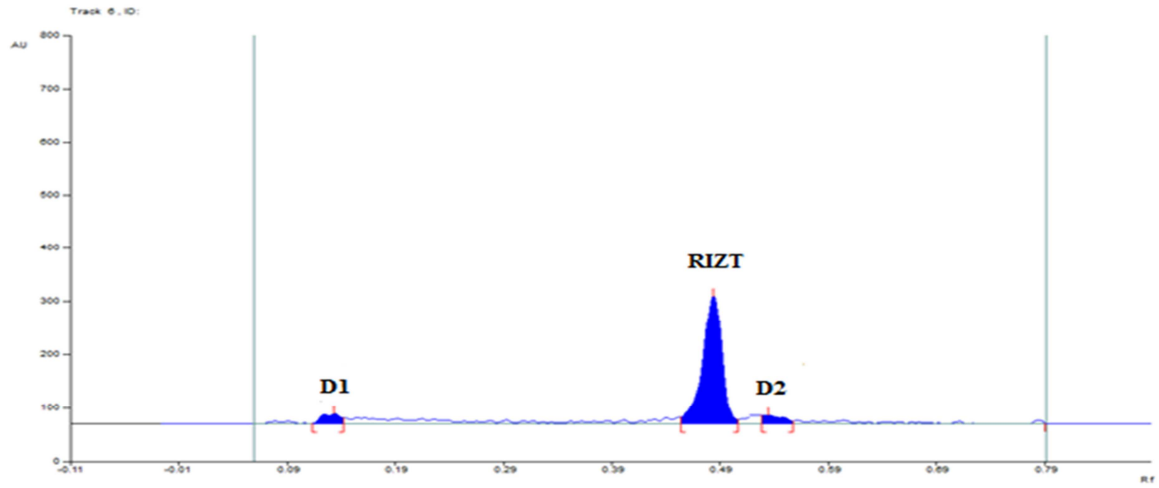

Fig. 1. Representative densitogram acid treated RIZT with degradation product D1(Rf = 0.13) and D2 (Rf = 0.53)

Alkali induced degradation

From working standard solution of RIZT (1000 ng μL^{-1}), 1 mL of solution was mixed with 1 mL of 0.1 N methanolicNaOH and 8 mL of methanol. The solution was kept at room temperature for 1 h and 15µL of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. The drug was found to be susceptible to alkali with 12.26 % of degradation. The representative densitogram after alkali treatment is shown in Figure 2.

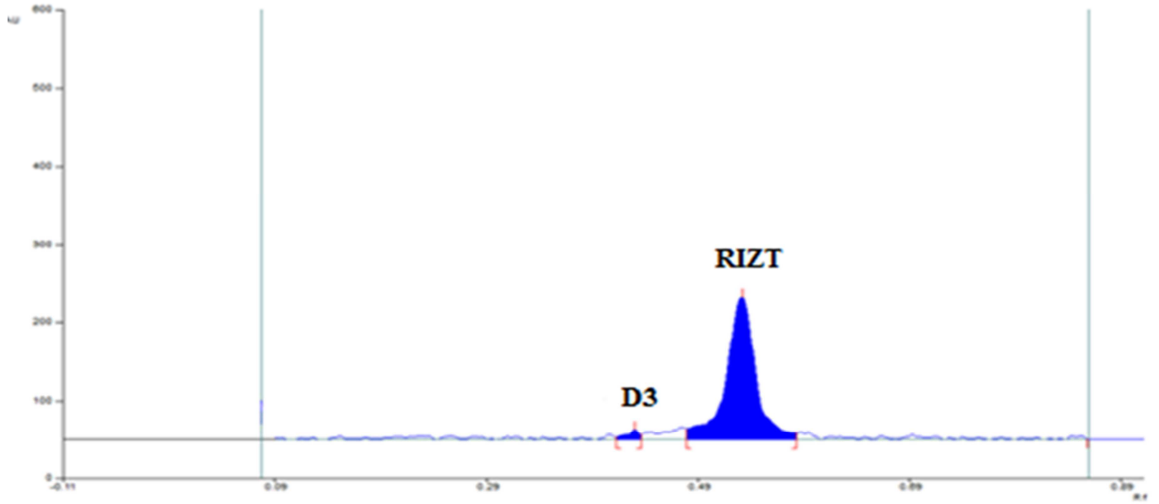

Fig. 2. Representative densitogram of alkali treated sample with degradation product D3 (Rf = 0.43)

Neutral Hydrolysis

1 mL working standard solution of RIZT (1000 ng μL^{-1}) was mixed with 1 mL of water and 8 mL methanol. The solution was kept at room temperature for 30 min and 15 µL of resulting solution was applied on TLC plate. 22.97 % degradation was observed without appearance of degradation product. The representative densitogram obtained after neutral degradation is shown in Figure 3.

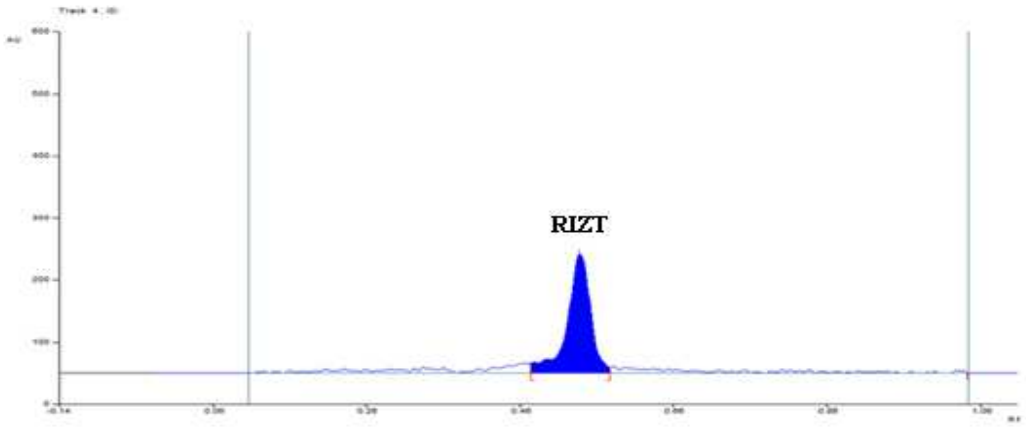

Fig. 3. Representative densitogram obtained after neutral degradation

Oxidative degradation

To 1 mL standard solution of RIZT (1000 ng μL^{-1}), 1 mL of 3 % solution of H_2O_2 and 8 mL of methanol was added and solution was kept at room temperature for 30 min. 15 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. Two additional degradation products with 26.62 % of degradation were observed when treated with 3 % H_2O_2. The representative densitogram after oxidative degradation is shown in Figure 4.

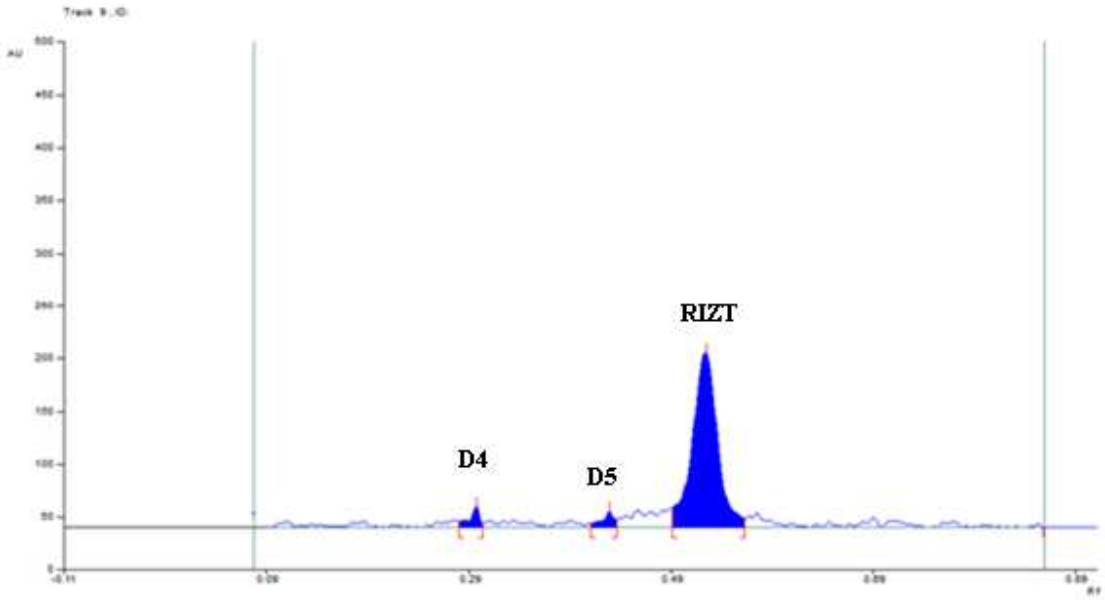

Fig. 4. Representative densitogram of sample subjected to oxidative degradation with degradation product D4 (Rf = 0.29) and D5 (Rf = 0.45)

Degradation under dry heat

Dry heat study was performed by keeping drug in oven at 60ºC for period of 8 h. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 100 ng μL^{-1}. 15 µL of the resulting solution was applied to HPTLC. 18.41 % of degradation was observed with additional peak at Rf value 0.68. The representative densitogram obtained from sample subjected to dry heat is shown in Figure 5.

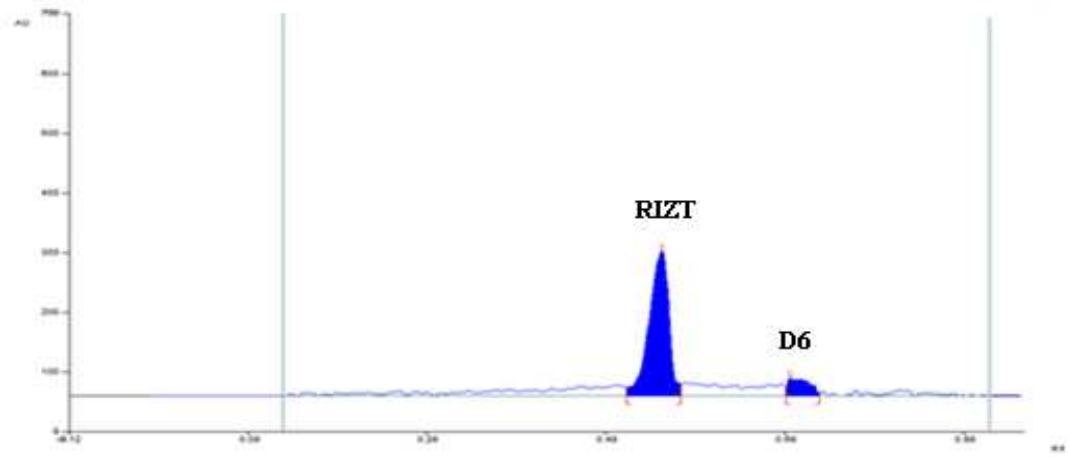

Fig. 5. Representative densitogram of sample subjected to dry heat with degradation product D6 (Rf = 0.68)

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main objective in developing this stability indicating HPTLC method is to achieve the resolution of RIZT and its degradation products. The chromatographic separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Benzene: Methanol (8:2, v/v) as mobile phase and detection was carried out at 227 nm. The retention factor for RIZT was found to be 0.50±0.006. Representative densitogram of standard solution of RIZT is shown in Figure 6.

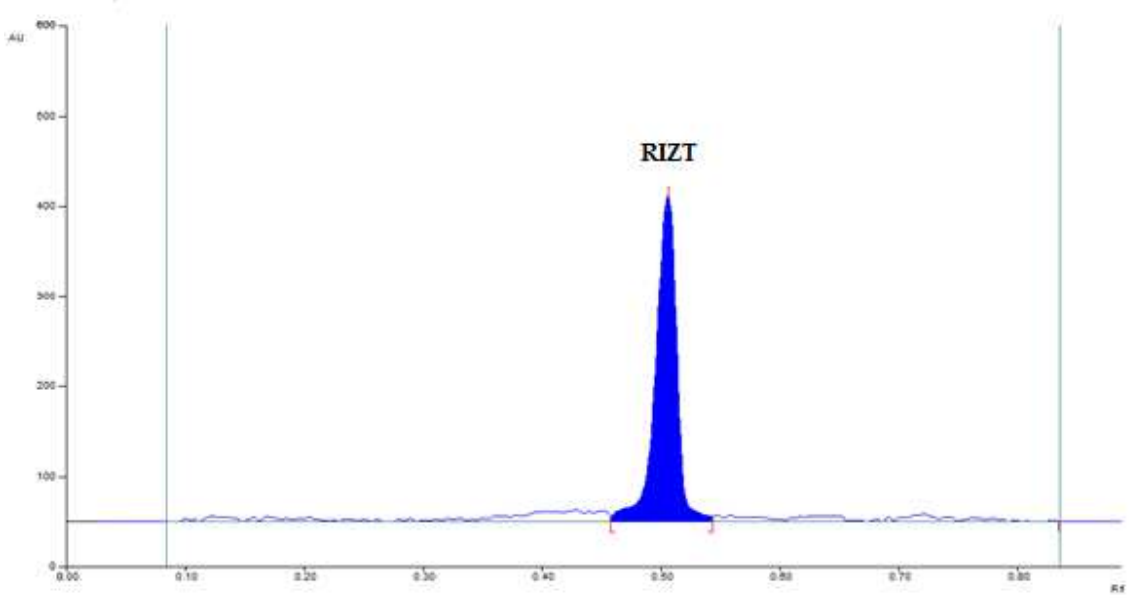

Fig. 6. Representative densitogram of standard solution of RIZT (2000 ngband^{-1}, Rf = 0.50 ± 0.006)

Result of forced degradation studies

Forced degradation study showed that the method is highly specific and there was no interference of degradation products observed at retention time of drug. Peak purity results greater than 995 indicate that drug peaks are homogeneous in all stress conditions tested. The unaffected assay of RIZT in the tablet confirms the stability indicating power of the method. The forced degradation studies data are summarized in Table 1.

Table 1. Data of forced degradation studies of RIZT

Stress conditions/ duration	% Assay of active substance	Rf values of degraded products
Acid/0.1 N HCl/ Kept at RT for 15min	75.35	0.13, 0.53
Alkali/0.1 N NaOH/ Kept atRT for 1 h	87.74	0.43
Oxidative /3 % H_2O_2 / Kept at RT for 30min	73.38	0.29, 0.45
Neutral/H_2O/ Kept at RT for 30min	77.03	---
Dry heat/ 60ºC/ 8 h	81.69	0.68

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines [9, 10].

Preparation of Calibration Curve

Aliquots of 1, 2, 3, 4 and 5 µL of standard solution of RIZT (500 ng μL^{-1}) were applied on TLC plate using Camag linomat sample applicator under nitrogen stream. TLC plates were dried, developed and densitometrically analyzed as described earlier. The linear regression data for calibration curves (n = 6) showed good linear relationship over a concentration range of 500-2500 ng $band^{-1}$ with high correlation coefficient > 0.991. The calibration curve obtained by plotting peak area against respective concentration is shown in Figure 7.

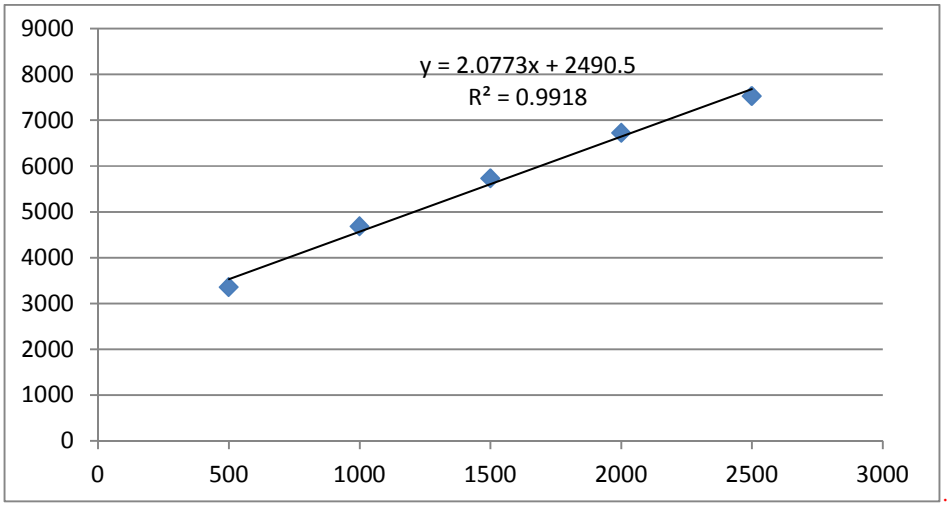

Fig. 7. Calibration curve for RIZT

Precision

Set of three different concentrations in three replicates of standard solutions of RIZT were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.22 to 0.31. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.36 to 0.61. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 99.90 ng $band^{-1}$ and 302.73 ng $band^{-1}$, respectively.

Recovery Studies

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 1000 ng $band^{-1}$from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies

indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are shown in Table 2.

Table 2. Recovery Studies of RIZT

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount found (ng band^{-1})	% Recovery	% RSD
RIZT	1000	800	1810.85	98.93	0.68
	1000	1000	2006.5	100.31	0.86
	1000	1200	2182.76	99.21	0.49

**Average of three determinations*

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 995, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, chamber saturation time was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results are given in Table 3.

Table 3. Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)
1	Mobile phase saturation (± 2 % methanol)	0.34
2	Chamber saturation time (± 10 %)	1.32

**Average of three determinations*

CONCLUSION

A new stability- indicating HPTLC method has been developed and validated for estimation of RIZT in bulk and tablet dosage form. The developed method was found to be simple, precise, accurate, and robust and it can be used for the routine analysis of RIZT in both bulk and tablet dosage forms. The forced degradation studies revealed suitability of the method to study stability of RIZT under various degradation conditions like acid, base, oxidative, thermal degradations.

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