Development and Validation of Stability Indicating HPTLC Method for Estimation of Rizatriptan Benzoate in Bulk and 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 the determination of Rizatiptan Benzoate in bulk and tablet formulation. Chromatographic separation was performed on aluminum plate precoated with Silica Gel 60 F254 using, Methanol: n-Propane: Triethylamine (3:5:2 v/v/v) as mobile phase followed by densitometric scanning at 280 nm. This system was found to give compact spot for Rizatiptan Benzoate (Rf value of Rf = 0.38 ± 0.02). The calibration curve was found to be linear between 800-2800 ng/band. The limit of detection and quantitation were found to be 2.24 ng/band and 6.79 ng/band respectively. The proposed method can be applicable for the estimation of Rizatiptan Benzoate during stability studies.

Keywords: Rizatiptan Benzoate, High Performance Thin Layer Chromatography (HPTLC), Stability-Indicating Method.

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Introduction

Rizatriptan benzoate is synthetically portrayed as: N, N-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)- 1H-indole-3-ethanamine (**Fig. 1**)[1]. It is a particular 5-hydroxyl triptamine 1B/1D receptor agonist to remember headache cerebral pains. Its experimental equation is C15H19N5 and its atomic weight is 269.34486 g/mol. Flow hypotheses on the etiology of migraine propose that indications are because of nearby cranial vasodilatation and/or to the arrival of vasoactive and professional provocative peptides from tactile nerve finishing in an enacted trigeminal framework.[2-4]

Since there are just couple of strategies are accessible for the determination of Rizatriptan benzoate. The present work is an endeavor to evaluate the same by a New HPTLC technique[5-7].

The writing audit demonstrates not very many techniques for the determination of Rizatriptan benzoate and pharmaceutical approvals by HPTLC strategy yet that different strategies like UV spectroscopic technique for Rizatriptan benzoate, HPLC technique for Rizatriptan benzoate, LC-MS and LC-MS/MS strategy for determination of Rizatriptan benzoate in human plasma[8].

This strategy can be effectively utilized for routine examination of Rizatriptan benzoate as it is fast, basic, specific and touchy strategy for the determination utilizing High Performance Thin Layer Chromatographic (HPTLC) system[9].

Materials and Methods

Instruments: The HPTLC system (Camag Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag Muttenz, Switzerland), twin-trough developing chamber (10 x 10cm), UV cabinet with dual wavelength UV lamps, Win-CATS software, syringe (100 µl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing.

Reagents and Materials: Rizatriptan Benzoate was received as a gift sample from Cipla Ltd., Mumbai. Methanol, chloroform, ammonia solution were of analytical grade (MERCK Chem. Ltd., Mumbai). Rizatriptan Benzoate formulation was purchased from local market.

Chromatographic Conditions: Chromatographic separation was performed on 10 × 10 cm aluminum backed plates pre-coated with 250 µm layer of silica gel 60 F254 (E. Merck, Darmstadt, Germany). The TLC plates were prewashed with methanol and dried in oven at 50^{0}C for 30 min. Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1µl/s. The TLC plate was developed in twin trough chamber using Methanol: n-Propanol: Triethylamine (3:5:2 v/v/v) as mobile phase at temperature, 25±2˚C; chamber saturation time, 15 min; migration distance, 75 mm. The TLC plate was scanned and analyzed by TLC Scanner IV and WinCATS software using following

parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 280 nm.

Preparation of standard solutions

Preparation of stock solution of standard rizatriptan benzoate: An accurately weighed RZT (20 mg) was transferred to 10 mL volumetric flask; dissolved in methanol and the volume was made up to mark with the same solvent to give 2000 ng/µL solution.

Preparation of working standard solution: The stock solution (0.5 ml) was withdrawn and transferred into 10 ml volumetric flask and diluted up to the mark with methanol (50µg/ml).

Preparation of Degradation solutions: Rizatriptan Benzoate was degraded in acid, alkaline, oxidative and photolytic stress conditions. The results are reported in **Table 1.**

Acid and base induced degradation: The 20 mg of RZT was separately dissolved in 10 ml of methanolic solution of 2N HCl and 2N NaOH. These solutions were reflux for 8 h room temperature. The 1ml of above solutions was taken and neutralized, then diluted up to 10 ml with methanol. The resultant solution were applied on TLC plate in triplicate (0.8 µl each, i.e. 1600 ng per spot). Chromatograph shown in **Fig. 3 and 4.**

Hydrogen peroxide – induced degradation: The 20 mg of RZT was separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (10.0%, v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on TLC plate in triplicate (0.8 µl each, i.e. 1600 ng per spot). Chromatograph shown in **Fig. 5.**

Dry heat degradation products: The powdered drug was kept at 60^0C for 8 h under dry heat condition and then 20 mg sample was taken and dissolved in 10ml methanol. Spot were applied and chromatograms were run.

Photo degradation products: The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 8 h. The resultant solution (0.8 µL, i.e. 1600 ng per spot) was applied on a TLC plate and chromatograms were run. Chromatograph shown in **Fig. 6.**

Preparation of Calibration curve: From working standard solution (400ng/ml) aliquots of 800, 1200, 1600, 2000, 2400 and 2800ng/mL were spotted on the TLC plate, dried, developed and analyzed. The calibration curve of peak area versus respective concentration was plotted and correlation coefficient and regression line equation were computed. Calibration curve shown in **Fig. 7.**

Validation of Developed HPTLC Method

Linearity: Linearity was performed using working standard of RZT. Calibration was done by applying standard stock solution ranging from 0.4-1.4 µL on TLC Plate; which gives concentration of 800-2800 ng/band. The plate was developed and scanned as described under above chromatographic conditions. Calibration curve was constructed by plotting the peak area vs. corresponding drug concentration. The results are reported in **Table 2** and the calibration curve in **Fig.7**. The 3-D linearity chromatogram is shown in **Fig. 8**.

Precision: Intraday precision was determined by analyzing, the three different concentrations 800 ng, 1200 ng and 1600 ng of RZT, for three times within the day. Day to day variability was assessed using above mentioned three concentrations and analyzing it for three consecutive days, which shows reproducibility of the method. Results are shown in **Table 3.**

Repeatability: Repeatability of sample application was assessed by applying 0.8 µL (1600 ng) of drug solution six times on a TLC plate followed by development of plate and recording the peak height and area for 6 bands **(Table 4)**.

Sensitivity: The limits of detection and quantification of the developed method were calculated from the standard deviation of the intercepts and mean slope of the calibration curves of Rizatriptan Benzoate using the formula as given below. LOD = 3.3 α/S LOQ = 10 α/S Where, α is the standard deviation of the intercepts of the five calibration curves and S is the mean slope of the five calibration curve. Results are shown in **Table 5**.

Accuracy: Accuracy was determined in terms of percent recovery. The proposed method was applied to determine Rizatriptan Benzoate in bulk and pharmaceutical dosage form. The recovery experiment was carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards at 80%, 100% and 120%. Preparation of solutions for accuracy study is given in **Table 6**.

Analysis of Pharmaceutical Dosage form: To determine the content of RZT in tablets, twenty tablets, each containing 5mg RZT, were accurately weighed and finely powdered. An amount equivalent to 20 mg RZT was transferred to 10 mL volumetric flask and extracted with methanol for 20 minutes by shaking mechanically. The solution was diluted to volume with the same solvent and filtered; from it, the sample solution (0.8 µL, containing 1600 ng of RZT) was

applied on TLC plate, developed and scanned. Results of the assay are shown in **Table 7**.

Analysis of Bulk Material: Accurately weighed quantity of 20 mg RZT was transferred to 10 mL volumetric flask, dissolved in methanol and volume was made up to mark. The solution (0.8 µL, containing 1600 ng of RZT) was applied; the plate was developed and scanned. The concentration was determined by regression equation and the results are shown in **Table 8.**

Results and Discussion

Selection of Wavelength: The working standard solution of rizatriptan benzoate (50µg/ml) was scanned between 200-800 nm and spectrum was recorded against methanol as blank in UV Visible spectrophotometer. The absorbance maximum (λmax) was found to be 280 nm which was selected as detection wavelength for rizatriptan benzoate.

Optimization of mobile phase: Numbers of trials were taken by changing the ratio of mobile phase with respect to achieve good resolution between rizatriptan benzoate and degradation products obtained in different degradation conditions with minimum tailing. The mobile phase Methanol: n-Propanol: Triethylamine (3:5:2 v/v/v) gave good resolution with Rf values of 0.38±0.02.

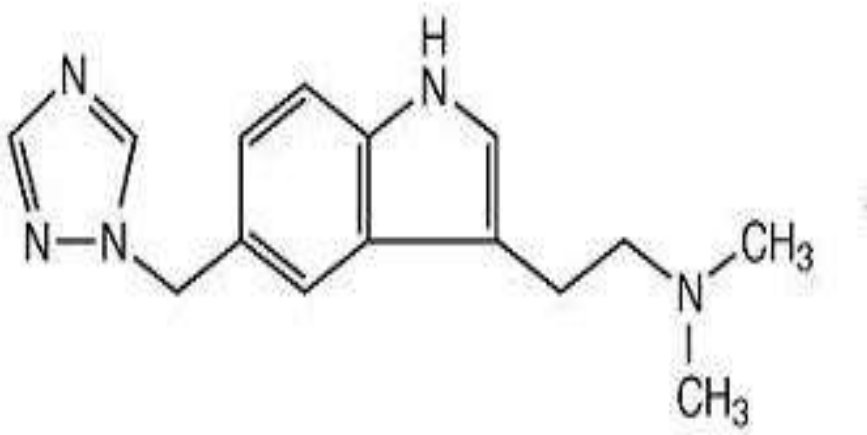

Fig. 1: Chemical structure of Rizatriptan Benzoate

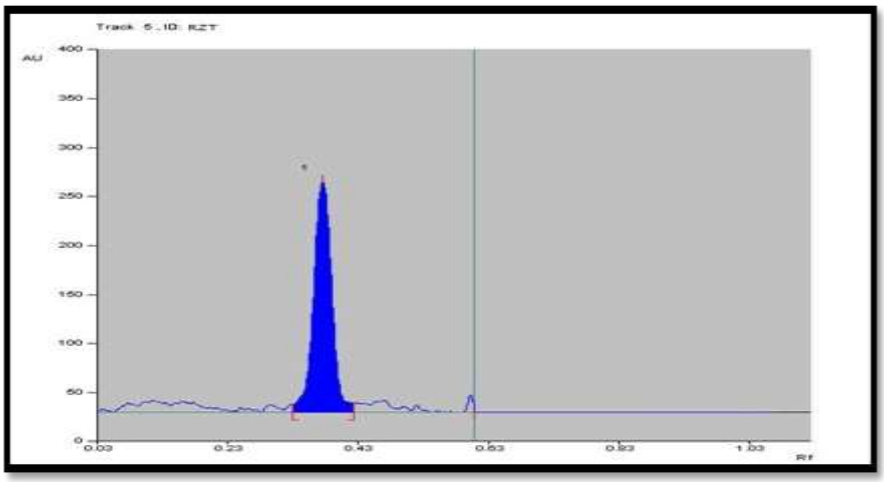

Fig. 2.a: TLC Chromatogram of RZT standard (R_f = 0.38 ± 0.02)

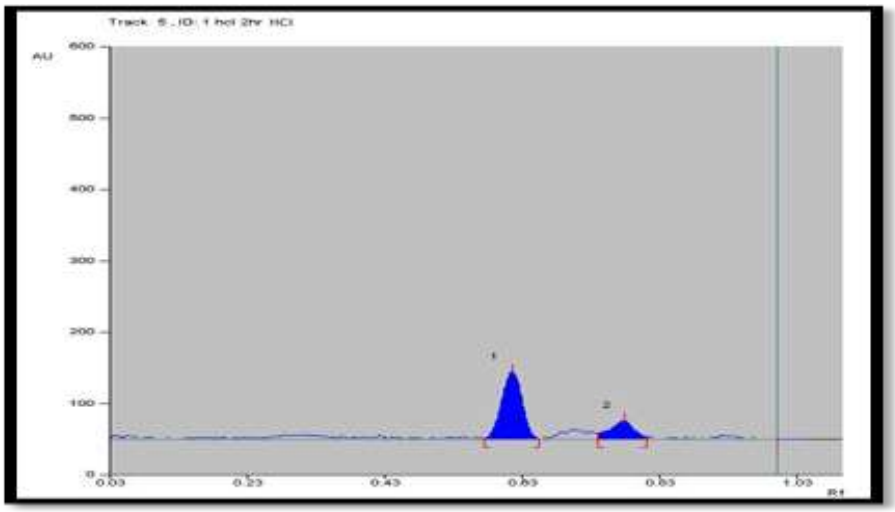

Fig. 3: Degradation of RZT in 2N HCl for 8hrs

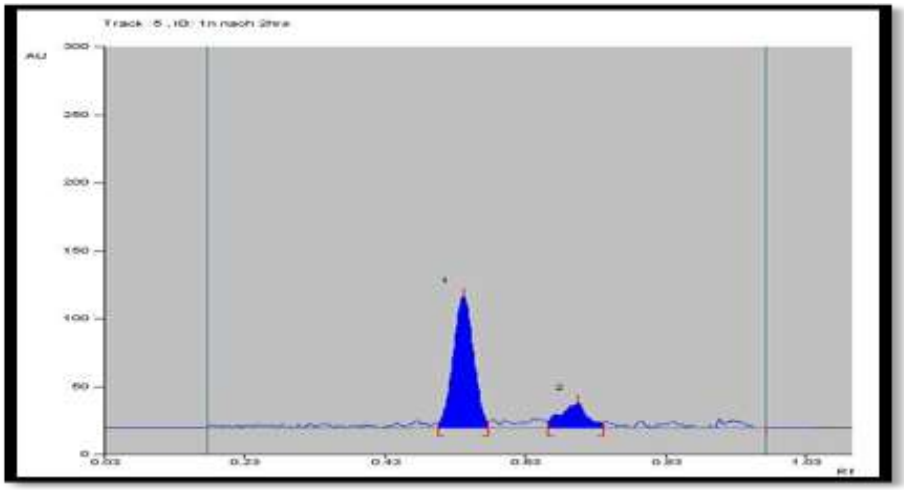

Fig. 4: Degradation of RZT in 2N NaOH for 8hrs

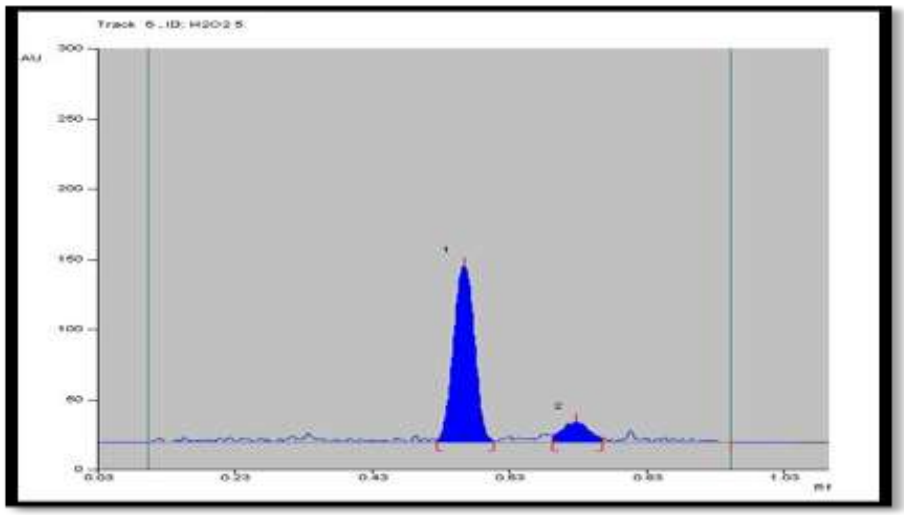

Fig. 5: Degradation of RZT in H_2O_2 for 8hrs

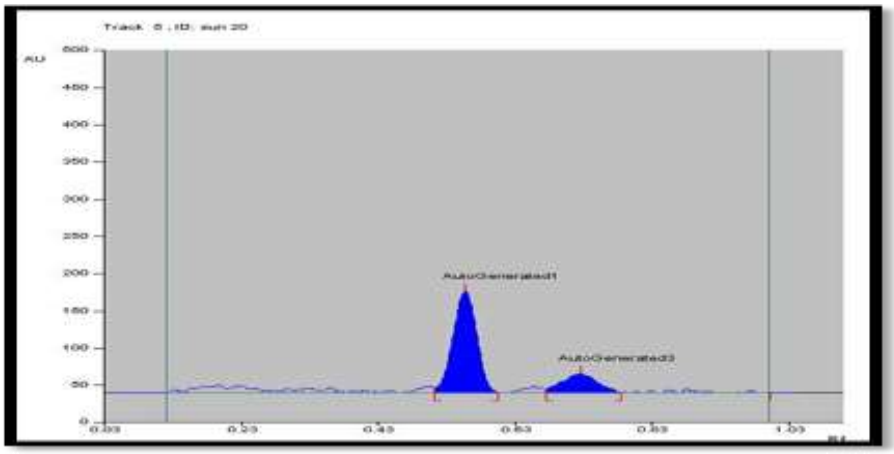

Fig. 6: Degradation of RZT in sun light for 8hrs

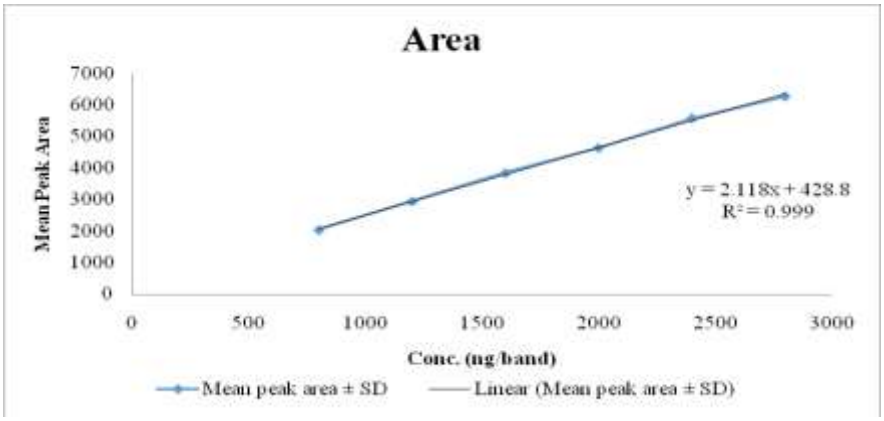

Fig. 7: Calibration curve of RZT

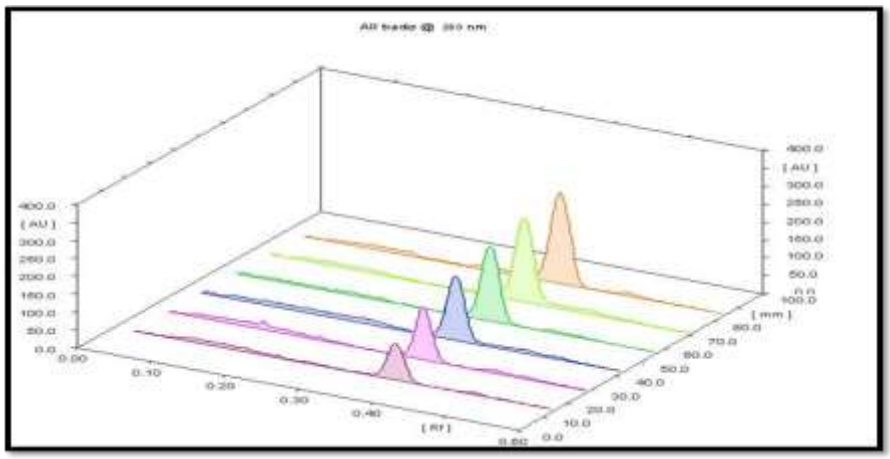

Fig. 8: 3-D linearity chromatogram of RZT

Table 1: Forced degradation studies

Reagent	Exposure time	Condition	Degradants peak	Degradants Rf	Drug Rf
1 N HCl	8 h	Room temp	peak Found	0.75	0.6
1 N NaOH	8 h	Room temp	peak Found	0.7	0.54
10% H_2O_2	8 h	Room temp	peak Found	0.69	0.54
Dry Heat	8 h	60^0 C	Not Found	-	-
Light	8 h	Sunlight	peak Found	0.72	0.55

Table 2: Linearity Study of RZT

Conc.(ng/band)	Mean peak area ± SD	% RSD
800	2035.15±52.11	2.560
1200	2936.57±58.77	2.920
1600	3837.75 ±20.78	0.5417
2000	4631.23±64.70	1.3970
2400	5571.51±42.14	0.7564
2800	6261.6±71.41	1.1405

Table 3: Data of Precision Study

Conc. (ng/band) (n=3)	Mean±SD	%RSD
800	1557.65±4.73	0.304
1200	2510.9±17.3	0.692
1600	3579.6±4.80	0.134

Table 4: Repeatability Study

Sr. no.	Conc. (ng/band)	Peak area ±SD
1	1600	4039.8
2	1600	4059.5
3	1600	4040.3
4	1600	4040.8
5	1600	4058.9
6	1600	4059.6
	mean±SD	4049.81±10.432
	% RSD	0.0183

Table 5: Sensitivity study

LOD (ng/band)	LOQ (ng/band)
2.24	6.79

Table 6: Recovery Study

Amount in %	Initial amount (ng) (n=3)	Amount added (ng/band)	% Recovered	% RSD
80	800	640	99.27	0.759
100	800	800	99.47	
120	800	960	100.68	

Table 7: Analysis of formulation of Tablets

Conc. (ng/band)	Amount found (ng/band)	% amount found
1600	1585.36	99.08
1600	1628.65	101
1600	1630.07	101
1600	1600.37	100
1600	1614.54	100.9
1600	1602.64	100.1
mean±SD	1610.27	100.34
% RSD	0.764	

Table 8: Results of Bulk Rizatriptan Benzoate

Conc. (ng/band)	Amount found (ng/band)	% amount found
1600	1612.8	100.8
1600	1570.2	98.1
1600	1577.9	98.6
1600	1593.9	99.6
1600	1608.6	100.5
1600	1582.2	98.8

mean±SD	1590.93±17.175	99.4
%RSD	0.997	

Conclusion

The proposed stability indicating HPTLC method for the estimation of Rizatriptan benzoate was precise, accurate, and selective. The methods were found to be linear in the concentration range of 800-2800 ng/spot. The developed method was free from interference of the excipients present in bulk and tablet dosage form. As the method could effectively separate the drug from their degradation products; it can be employed as a stability indicating one.

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