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Development and Validation of Stability Indicating HPTLC Method for Estimation of Rizatriptan Benzoate in Bulk and Tablet Dosage Form

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Abstract

For the determination of rizatriptan benzoate in bulk and tablet formulation, a novel, straightforward, accurate, precise, and selective stability-indicating high performance thin layer chromatographic (HPTLC) approach has been created and validated. Using methanol: n-propane:triethylamine (3:5:2 v/v/v) as the mobile phase and densitometric scanning at 280 nm, chromatographic separation was carried out on an aluminum plate that had been precoated with Silica Gel 60 F254. Rizatriptan benzoate was shown to have a compact spot in this system (R_f value of $R_f = 0.38 \pm 0.02$). It was discovered that the calibration curve was linear between 800-2800 ng/band. It was discovered that the limits of detection and quantitation were 2.24 ng/band and 6.79 ng/band, respectively. During stability investigations, the suggested approach may be used to estimate Rizatriptan Benzoate.

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

Introduction

Fig. 1 shows the synthetic representation of rizatriptan benzoate as N, N-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine. [1]. To recall headache brain aches, a specific 5-hydroxyl triptamine 1B/1D receptor agonist is used. It weighs 269.34486 g/mol and has the experimental formula $C_{15}H_{19}N_5$. According to flow theories about the origin of migraine, symptoms may be caused by localized cranial vasodilatation or by the arrival of professional stimulating and vasoactive peptides from tactile nerve finishing in an activated trigeminal framework. [2-4] There are just a few methods available for determining rizatriptan benzoate. The goal of the current work is to assess the same using a novel HPTLC technique [5-7]. The writing audit shows that there aren't many methods for determining Rizatriptan benzoate and pharmaceutical approvals using HPTLC strategy; however, there are several methods, such as UV spectroscopic method, HPLC method, LC-MS, and LC-MS/MS method for determining Rizatriptan benzoate in human plasma [8]. Because it is quick, straightforward, precise, and sensitive, this method can be used successfully for routine Rizatriptan benzoate examinations using a method called High Performance Thin Layer Chromatography (HPTLC) [9].

Materials

and

Procedures

Instruments: For chromatographic analysis, the HPTLC system (Camag Switzerland) comprising the Linomat V semiautomatic spotting device, TLC Scanner IV (Camag Muttentz, Switzerland), twin-trough

developing chamber (10 x 10 cm), UV cabinet with dual wavelength UV lamps, Win-CATS software, and syringe (100 µl capacity, Hamilton) was utilized. All of the weighing was done using an electronic analytical balance (Shimadzu AUX-220).

Materials and Reagents: Cipla Ltd., Mumbai, provided a gift sample of rizatriptan benzoate. Ammonia solution, methanol, and chloroform were analytical grade (MERCK Chem. Ltd., Mumbai). The formulation of rizatriptan benzoate was bought from the neighborhood market. Chromatographic Conditions: 10 x 10 cm aluminum-backed plates that had been previously covered with a 250 µm layer of silica gel 60 F254 (E. Merck, Darmstadt, Germany) were used for chromatographic separation. After being prewashed with methanol, the TLC plates were dried for 30 minutes at 50°C in the oven. The following parameters were used by the Linomat V semi-automatic spotter to spot samples on TLC plates 15 mm from the bottom edge: Application rate: 0.1µl/s; track distance: 11.6 mm; band width: 6 mm. Using Methanol: n-Propanol: Triethylamine (3:5:2 v/v/v) as the mobile phase, the TLC plate was created in a twin trough chamber at 25±2°C, with a migration distance of 75 mm and a chamber saturation period of 15 minutes. The TLC Scanner IV and WinCATS software used the following to scan and analyze the TLC plate:

parameters: detecting wavelength: 280 nm; scanning speed: 20 mm/sec; slit size: 4 × 0.30 mm. Getting standard solutions ready Making a standard rizatriptan benzoate stock solution: To create a 2000 ng/µL solution, a precisely weighed RZT (20 mg) was put into a 10 mL volumetric flask, dissolved in methanol, and the volume was adjusted to mark using the same solvent. The working standard solution was prepared by removing 0.5 ml of the stock solution, transferring it into a 10 ml volumetric flask, and diluting it with 50µg/ml of methanol. Degradation solution preparation: Under acidic, alkaline, oxidative, and photolytic stress conditions, rizatriptan benzoate was broken down. Table 1 presents the findings. Degradation caused by acids and bases: Ten milliliters of a methanolic solution of 2N HCl and 2N NaOH were used to individually dissolve the 20 mg of RZT. These solutions were allowed to reflux at room temperature for eight hours. After neutralizing 1 milliliter of the aforementioned solutions, they were diluted to Ten milliliters of methanol. The final solution (0.8 µl each, or 1600 ng each spot) was applied in triplicate on a TLC plate. Chromatographs are displayed in Figures 3 and 4. Degradation caused by hydrogen peroxide: 20 mg of RZT were separately dissolved in 10 ml of a hydrogen peroxide methanolic solution (10.0%, v/v). To rule out the potential degradative influence of light, the solution was left at room temperature in the dark for eight hours. Three separate applications of the resulting solution (0.8 µl each, or 1600 ng per spot) were made on a TLC plate. The chromatogram in Figure 5.

Dry heat degradation products: A 20 mg sample was obtained and dissolved in 10 ml of methanol after the powdered medication was heated to 600C for eight hours. Chromatograms were done after spots were applied. Photo degradation products: By subjecting the stock solution to direct sunshine for eight hours, the drug's photochemical stability was also investigated. Chromatograms were performed when the resulting solution (0.8 µL, or 1600 ng per spot) was put onto a TLC plate. The chromatogram in Figure 6. Calibration curve preparation: 800, 1200, 1600, 2000, 2400, and 2800 ng/mL aliquots were spotted on the TLC plate, dried, developed, and examined from the working standard solution (400 ng/mL). Plotting of the peak area calibration curve against the corresponding concentration was done, and the correlation coefficient as well as the regression line equation. The calibration curve is displayed in Figure 7. Verification of the Developed HPTLC Technique Linearity: RZT's working standard was used to perform linearity. A standard stock solution with a concentration of 800-2800 ng/band and a volume range of 0.4-1.4 µL was applied to the TLC plate for calibration. Under the chromatographic conditions mentioned above, the plate was developed and scanned. Plotting the peak area against the relevant drug concentration allowed for the construction of the calibration curve. Table 2 reports the results, and Fig. 7 shows the calibration curve. Fig. 8 displays the 3-D linearity chromatogram.

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Precision: By examining the three distinct concentrations of RZT—800 ng, 1200 ng, and 1600 ng—three times during the day, intraday precision was ascertained. Using the three concentrations indicated above, day-to-day variability was measured and analyzed for three days in a row, demonstrating the method's reproducibility. Table 3 displays the results.

Repeatability: To evaluate the repeatability of sample application, 0.8 μ L (1600 ng) of drug solution was applied six times on a TLC plate. The plate was then developed, and the peak height and area for each of the six bands were recorded (Table 4).

Sensitivity: Using the formula below, the limits of detection and quantification of the devised method were determined using the mean slope of the Rizatriptan Benzoate calibration curves and the standard deviation of the intercepts. LOD equals $3.3 \alpha/S$. LOQ is $10 \alpha/S$. where S is the average slope of the five calibration curves and α is the standard deviation of the intercepts. Table 5 displays the results.

Accuracy: The percentage of recovery was used to calculate accuracy. Rizatriptan benzoate was measured in pharmaceutical dose form and in bulk using the suggested method. Three distinct standard concentrations—80%, 100%, and 120%—were spiked into previously examined samples in triplicate to conduct the recovery experiment. Table 6 contains the solutions prepared for the accuracy investigation.

Pharmaceutical Dosage Form Analysis: Twenty tablets, each containing 5 mg of RZT, were precisely weighed and ground into a fine powder in order to ascertain the amount of RZT present. A 10 mL volumetric flask containing 20 mg of RZT was filled with methanol and shaken mechanically for 20 minutes. After using the same solvent to dilute the solution to volume and filtering it, the sample solution (0.8 μ L, comprising 1600 ng of RZT) was applied, developed, and scanned on a TLC plate. Table 7 displays the assay's results.

Examination of Bulk Material: 20 mg of precisely weighed RZT was added to a 10 mL volumetric flask, dissolved in methanol, and the volume was adjusted to the mark. After applying the solution (0.8 μ L, containing 1600 ng of RZT), the plate was developed and scanned. Table 8 displays the results of the regression equation used to determine the concentration.

Findings and Conversation
Wavelength Selection: The rizatriptan benzoate working standard solution (50 μ g/ml) was scanned.

The UV Visible Spectrophotometer was used to record the spectra between 200 and 800 nm using methanol as a blank. Rizatriptan benzoate was discovered to have an absorbance maximum (λ_{max}) of 280 nm, which was chosen as the detection wavelength.

Mobile phase optimization: Several experiments were conducted by varying the mobile phase ratio in order to produce minimal tailing and good resolution between rizatriptan benzoate and degradation products obtained under various degradation settings. Methanol is the mobile phase: -R_f values of 0.38 ± 0.02 indicated good resolution for propanol:triethylamine (3:5:2 v/v/v).

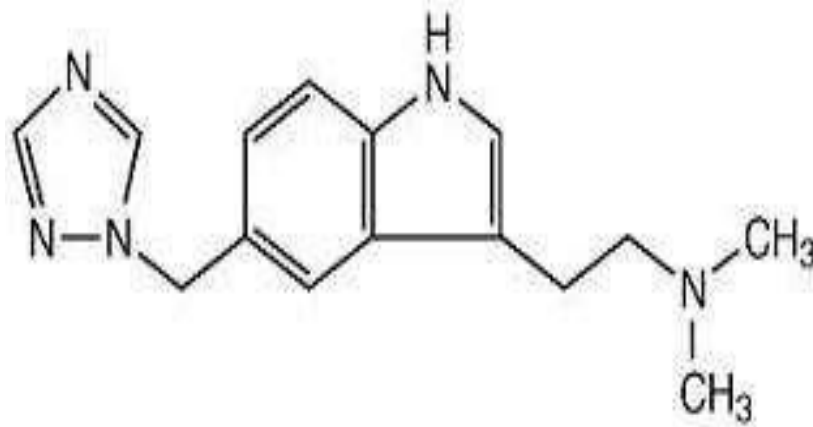


Fig. 1: Chemical structure of Rizatriptan Benzoate

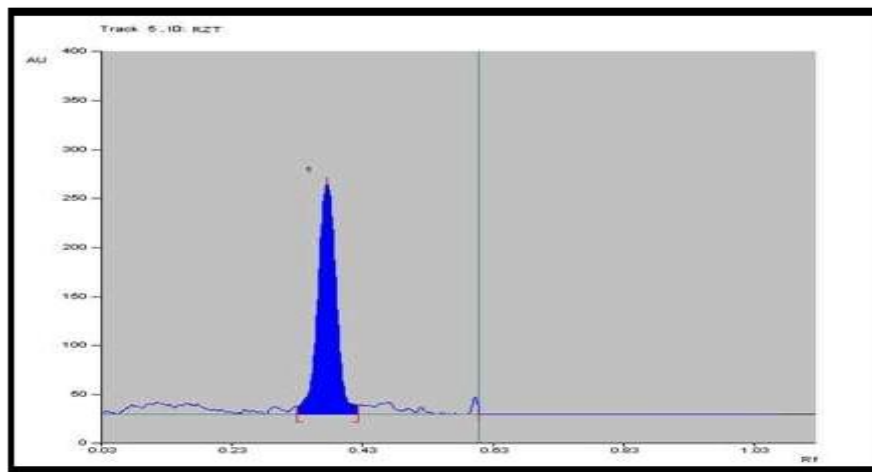


Fig. 2.a: TLC Chromatogram of RZT standard ($R_f = 0.38 \pm 0.02$)

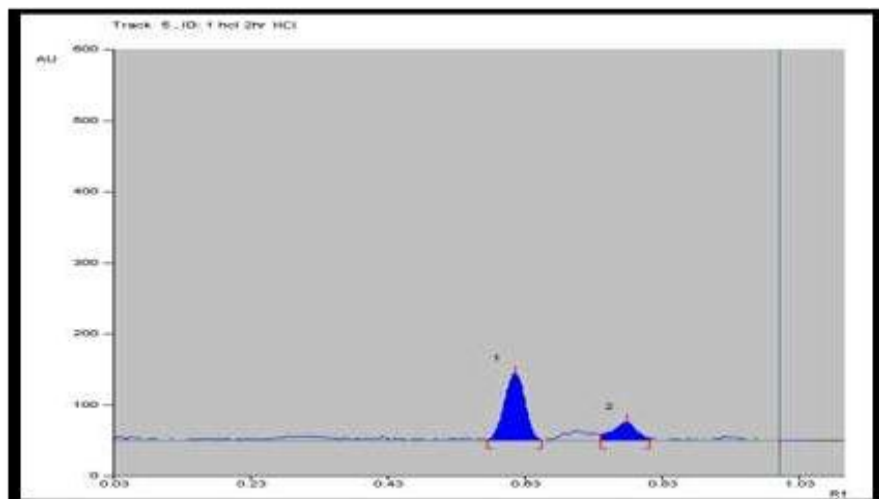


Fig. 3: Degradation of RZT in 2N HCl for 8hrs

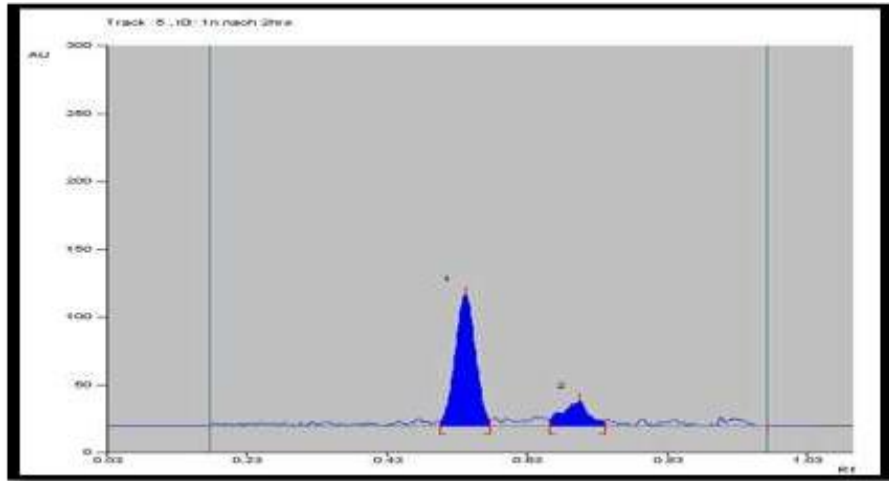


Fig. 4: Degradation of RZT in 2N NaOH for 8hrs

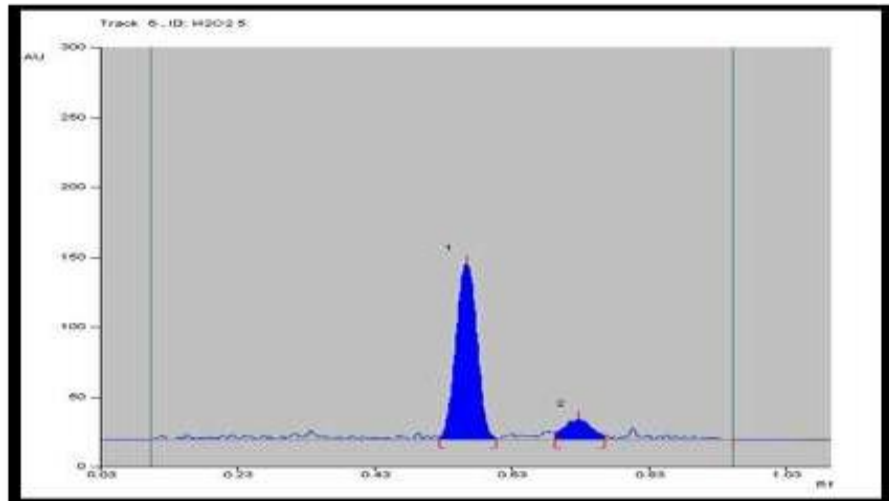


Fig. 5: Degradation of RZT in H₂O₂ for 8hrs

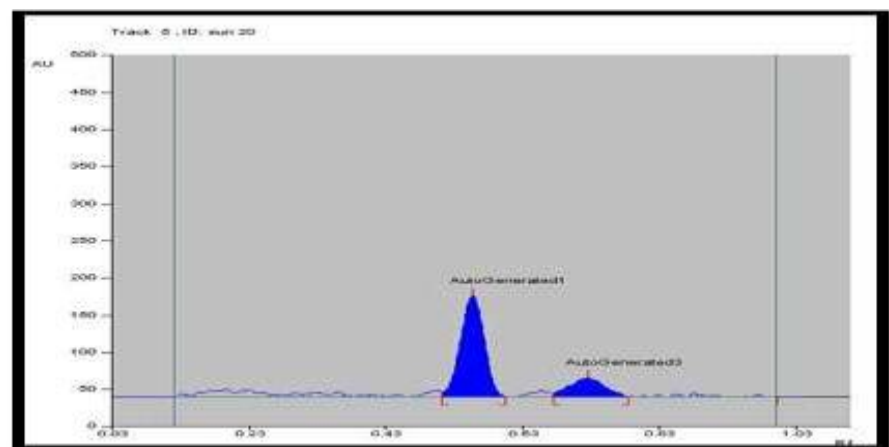


Fig. 6: Degradation of RZT in sun light for 8hrs

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 ₂ O 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

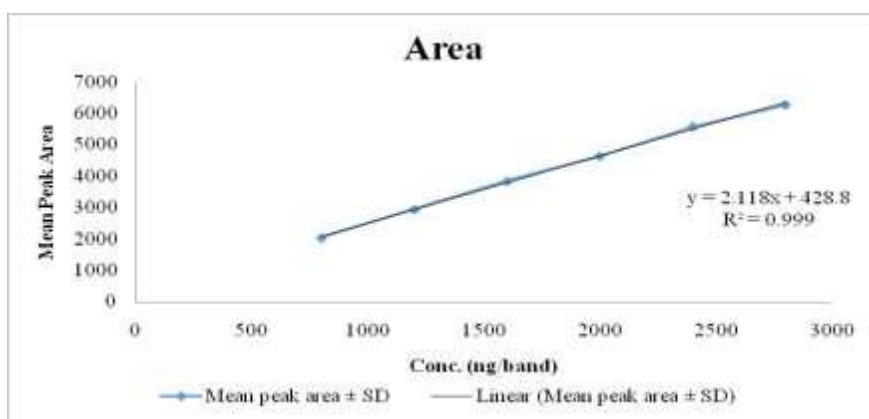


Fig. 7: Calibration curve of RZT

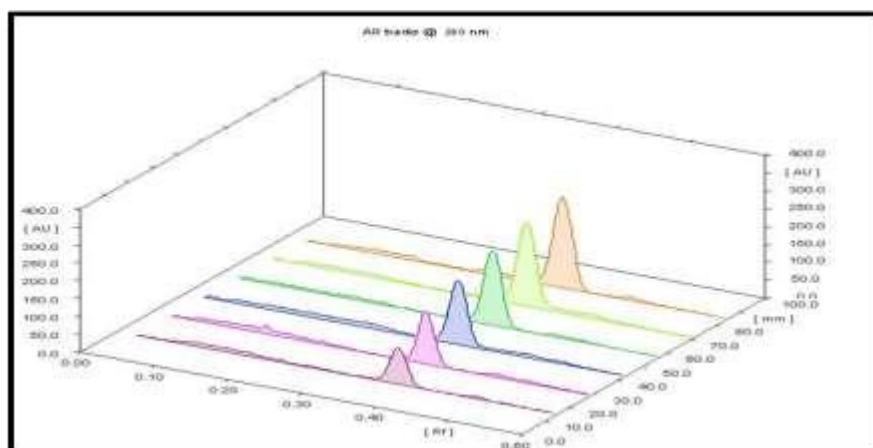


Fig. 8: 3-D linearity chromatogram of RZT

Table 1: Forced degradation studies

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	

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Conclusion

Rizatriptan benzoate was precisely, accurately, and selectively estimated using the suggested stability-indicating HPTLC approach. It was discovered that the techniques were linear in the 800–2800 ng/spot concentration range. The excipients used in tablet and bulk dose forms did not interact with the established technique. The technique can be used as a stability indicator since it can successfully separate the medication from its degradation products.

Recognitions

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