ORIGINAL ARTICLE

Development and Validation of Stability-indicating Analytical Method for a Multiparticulate Drug Delivery System Containing Rizatriptan Benzoate

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Abstract

A stability-indicating analytical method was developed for a newly formulated multiparticulate drug delivery system containing rizatriptan benzoate that is meant for administration by the sublingual route. A forced degradation study was performed to determine the breakdown products of rizatriptan benzoate. Subsequently, a high-performance liquid chromatography (HPLC)-based analytical method was developed to estimate the shelf-life of a multiparticulate drug delivery system. The HPLC parameters were deliberately varied to validate the robustness and ruggedness of the analytical method. This validated method was then used to propose the shelf-life of a newly formulated drug delivery system of rizatriptan benzoate stored at 40 ± 2°C and 75 ± 5% RH for 6 months. Forced degradation studies revealed significant degradation under acidic, oxidative, and photolytic conditions, while the drug was stable under neutral and thermal stress. The developed HPLC method successfully separated the drug from its degradation products with good resolution. The developed HPLC method qualified for accuracy, precision, specificity, linearity, robustness, and sensitivity. Validation studies demonstrated accuracy of 98.6%, precision (RSD <2%), linearity (R^2 = 0.999), and robustness. The results of the stability study indicated that the formulation did not exhibit significant changes when packed in paper sachets and stored at accelerated stability conditions for 6 months. Therefore, it is proposed that the multiparticulate drug delivery system containing rizatriptan benzoate, when packed in paper sachets, can be stored for a period of up to 24 months at room temperature. However, the results need to be confirmed by performing real-time stability studies in future work.

Key words: Rizatriptanbenzoate, forced degradation, stability-indicating method, method validation, pharmaceutical analysis

INTRODUCTION

Rizatriptan benzoate, an antimigraine medication of the triptan family, preferentially agonistically binds to the 5-hydroxytryptamine (5-HT_1B/1D) receptor. It is frequently used to treat adult migraine headaches, including aura, right away. The drug efficiently reduces migraines by narrowing cerebral arteries and preventing the synthesis of neuropeptides due to its high affinity for 5-HT_1B and 5-HT_1D receptors.[1-3]

A development of stability-indicating analytical methods is crucial for pharmaceutical quality control, as mandated by regulatory agencies worldwide. Even in the presence of impurities, excipients, and degradation products, these methods must be able to quantify the active pharmaceutical ingredient correctly.[4-6] The International Council for Harmonization's (ICH) Q2(R1) and Q1A(R2) guidelines offer thorough frameworks for stability testing and analytical technique validation.[7,8]

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Several analytical techniques, such as spectrophotometric,[9] high-performance thin-layer chromatography,[10] and liquid chromatography methods, have been employed to determine rizatriptan benzoate.[11-13] Few research, nonetheless, have concentrated on creating thorough stability-indicating techniques with in-depth forced degradation analyses. This approach attempts to close this gap by creating a strong and high-performance liquid chromatography (HPLC) method that can distinguish rizatriptan benzoate from its likely breakdown products under various stress conditions.

The main objectives of this study were to perform thorough forced degradation investigations, assess the long-term stability profile under accelerated conditions, and develop and validate a stability-indicating HPLC procedure for the quantitative prediction of rizatriptan benzoate in pharmaceutical granules. By offering a trustworthy technique for rizatriptan benzoate formulation quality control and stability evaluation, this work advances the field of pharmaceutical analytical sciences.

MATERIALS AND METHODS

Chemicals and reagents

Rizatriptan benzoate relative standard (purity >99%) was procured from a certified company Zim Laboratories Limited (Nagpur). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Analytical grade potassium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained using a Millipore water purification system (Millipore, Bedford, MA, USA). All chemicals and solvents were of analytical or HPLC grade.

Forced degradation studies of the drug

Rizatriptan benzoate (equivalent to 10 mg) was subjected to different stress conditions to evaluate its degradation behavior. For acidic hydrolysis, the drug was treated with 1 N hydrochloric acid at 60°C for 20 min, cooled, neutralized with 1 N sodium hydroxide, filtered, and analyzed by HPLC. In basic hydrolysis, it was treated with 1 N sodium hydroxide at 60°C for 20 min, cooled, neutralized with 1 N hydrochloric acid, filtered, and analyzed by HPLC. Oxidative degradation was carried out by treating the drug with 50% hydrogen peroxide at 60°C for 5 min, followed by filtration and HPLC analysis. For thermal degradation, the powdered drug was exposed to dry heat at 105°C for 30 h, filtered, and analyzed by HPLC. Photolytic degradation was performed by spreading the powdered sample in a petri plate and exposing it for a duration enough to achieve visible light (1.2 million lux hours) and ultraviolet (UV) light (200 watt-hours/m^2). The chromatogram of rizatriptan benzoate in mobile phase exhibited peaks at 4.1, 7.3 minutes. The stability of this rizatriptan solution under several stress conditions was determined by forced degradation studies [Figure 1].

Instrumentation

A UV-visible detector, quaternary pump column oven, and autosampler made up the HPLC system. Chromatography instruments were used for data collection and processing. A pH meter that had been calibrated was used to measure the pH. The analytical balance used for the weighing has a readability of 0.1 mg.

Chromatographic conditions

The optimized chromatographic conditions were as follows: Column: C18, measuring 250 mm by 4.6 mm and 5 µm, Acetonitrile (70:30, v/v) and phosphate buffer (pH 3.0) comprise the mobile phase.

1.0 mL/min flow rate, 225 nm detection wavelength, 20 µL injection volume, 30°C column temperature, and 10 min run time.

Preparation of standard solutions

By dissolving 10 mg of the standard for reference in 10 mL of mobile phase, an initial solution of rizatriptan benzoate (1,000 µg/mL) has been produced. By diluting the stock solution with the mobile phase, working standards were prepared, yielding concentrations between 50 and 150 µg/mL.

Sample preparation

Granules equivalent to 10 mg of rizatriptan benzoate were accurately weighed and dissolved in the mobile phase and sonicated for 10 min, final dilution to 100 mL, then filtered through 0.45 mm filter.

Analytical method development of the drug

The analytical method development for Rizatriptan benzoate (equivalent to 10 mg) was carried out using an RP-HPLC method. Rizatriptan benzoate was measured at a reported wavelength of 225 nm using Photo diode array detector. Initial trials were performed with C18 column (150 × 4.6 mm, 5 µm) using a mobile phase consisting of phosphate buffer pH 3.0 (0.02 M, pH 3.0) and acetonitrile in the ratio of 70:30%v/v at a flow rate of 1 mL/min in isocratic mode for assay determination. The column temperature was maintained at 30°C, and injection volume was 20 µL to obtain a peak of Rizatriptan benzoate with a retention time of about 3–5 min.

Analytical method validation of sublingual granules

The developed HPLC method for rizatriptan benzoate was validated to ensure its reliability for the analysis of

rizatriptan benzoate granules. System suitability was verified by evaluating replicate injections of the standard solution, considering parameters such as tailing factor, theoretical plates, retention time, and relative standard deviation. Specificity was demonstrated through the use of forced degradation samples, confirming the method's ability to separate the analyte from potential degradants. Linearity was established across 50–150% of the assay concentration (in triplicate), with the correlation coefficient (R^2) determined from the calibration curve of peak area versus concentration. Accuracy was evaluated through recovery studies at 80%, 100%, and 120% levels, where known amounts of rizatriptan benzoate were spiked into the sample matrix and recovery percentages were calculated. Precision was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day). Repeatability was tested using six replicate injections on the same day, while intermediate precision was confirmed by analyses conducted over three different days. Sensitivity was determined by calculating the limit of detection (LOD) and limit of quantitation (LOQ) using the signal-to-noise ratio - 3:1 and 10:1 corresponded to LOD and LOQ, respectively. Robustness was evaluated by introducing deliberate variations in chromatographic conditions, including minor adjustments to detection wavelength (±2 nm), mobile phase composition (±5%), and flow rate (±0.1 mL/min), demonstrating the method's reliability under slight changes.

Accelerated stability studies of sublingual granules

The accelerated stability study of rizatriptan benzoate granules was carried out to evaluate the effect of elevated temperature and humidity on product quality. Representative samples from batches were packed in aluminium sachets and placed in stability chambers maintained at 40 ± 2°C and 75 ± 5% RH. The samples were withdrawn at 0, 1, 3, and 6 months and analyzed for physical appearance, assay, moisture content, and pH. Robustness of the packaging and product performance was assessed by comparing results with initial values and established acceptance criteria.

RESULTS AND DISCUSSION

Forced degradation studies of the drug

The stability of rizatriptan benzoate under several stress conditions was determined by forced degradation studies:

Acidic hydrolysis

The chromatogram exhibited multiple peaks (at 3.9, 7.2 min) within 15 min, indicating distinct degradation of rizatriptan benzoate in an acidic environment. The rizatriptan benzoate degraded by 5.20% in an acidic environment [Figure 2].

Basic hydrolysis

The chromatogram exhibited multiple peaks (at 3.9, 7.2 min) within 15 min, indicating distinct degradation of rizatriptan benzoate in a basic environment. The rizatriptan benzoate degraded by 2.81% in a basic environment [Figure 3].

Oxidative degradation

The chromatogram exhibited multiple peaks (at 3.9, 7.1 min) within 15 min, indicating distinct degradation of rizatriptan benzoate in the presence of hydrogen peroxide. No co-elution with the primary peak was confirmed by peak purity analysis. The rizatriptan benzoate degraded by 3.79% in the presence of oxidative stress [Figure 4].

Thermal degradation

The chromatogram exhibited multiple peaks (at 3.9, 7.1 min) within 15 min, but the secondary peak was insignificant, indicating low degradation of rizatriptan benzoate in the presence of dry heat [Figure 5].

Photolytic degradation

The chromatogram exhibited multiple peaks (at 3.9, 7.2 min) within 15 min, indicating distinct degradation of rizatriptan benzoate in the presence of UV-visible light. The rizatriptan benzoate degraded by 1.43% in the presence of light [Figure 6].

Analytical method development of the drug

Chromatographic conditions were optimized to develop a stability-indicating HPLC method for rizatriptan benzoate. Standard and sample solutions were prepared in buffer: acetonitrile as diluent, maintaining an assay concentration range of 50–150 µg/mL. The optimum chromatographic performance was achieved using phosphate buffer at pH 3.0 mixed with acetonitrile in a 70:30 (v/v) ratio. The detection wavelength was 227 nm, though the maximum absorbance of pure rizatriptan benzoate was observed at 225 nm; this provided adequate sensitivity while minimizing interference from degradation products and excipients. Under the optimized conditions, the drug eluted at 4.5 min with 4,200 theoretical plates, a tailing factor of 1.1, %RSD of 0.32, and good peak resolution. Method specificity was demonstrated through forced degradation studies, which confirmed effective separation of rizatriptan benzoate from its degradation products, while PDA analysis verified peak purity. Robustness testing, performed by varying flow rate (±0.1 mL/min), mobile phase composition (±5%), and wavelength (±2 nm), confirmed that the method remained unaffected by small deliberate changes. Both standard and sample solutions were stable for up to 24 h.

Analytical method validation of sublingual granules

System suitability

Throughout the validation, the system suitability parameters – theoretical plates >3,000, tailing factor <2, and RSD <2% for replicate injections – consistently achieved the acceptance parameters.

Specificity

The method demonstrated excellent specificity, with no interfering peaks observed at the retention time of rizatriptan benzoate 4.5 min [Figures 7-10]. The non-existence of interference from excipients and other matrix components was verified by the chromatograms of blank samples, standard solutions, and sample solutions.

Linearity and range

With a correlation coefficient (R^2) of 0.999, the technique demonstrated exceptional linearity over the concentration range of 50–150 µg/mL. The method's capacity to produce consistent statistical results across the tested range is demonstrated by the linearity statistics, which are shown in Table 1.

Accuracy data

Across the three examined levels, recovery studies showed due accuracy, with mean recovery values ranging from 98.6% to 101.8%. Table 2 displays the comprehensive recovery data, which demonstrates the method's accuracy in quantifying rizatriptan benzoate when excipients are present.

Precision

RSD values for both intra-day and inter-day precision experiments were well below 2%, demonstrating the method's exceptional precision. Table 3 summarizes the precision statistics, demonstrating the repeatability and dependability of the procedure.

LOD and quantitation

It was found that the LOD and LOQ values were 0.05 µg/mL and 0.15 µg/mL, respectively, indicating the sensitivity of the method employed for trace-level measurement.

Robustness

The technique demonstrated resilience to intentional changes in chromatographic conditions. The method's performance was not substantially impacted by changes in flow rate,

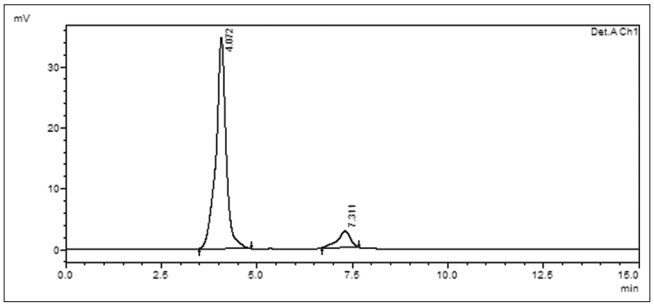

Figure 1: Standard rizatriptan benzoate for force degradation

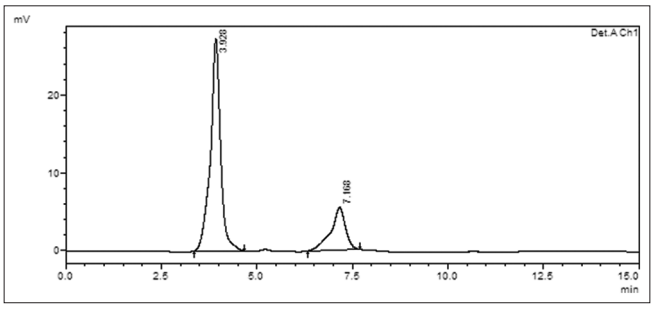

Figure 2: Force degradation-acidic hydrolysis

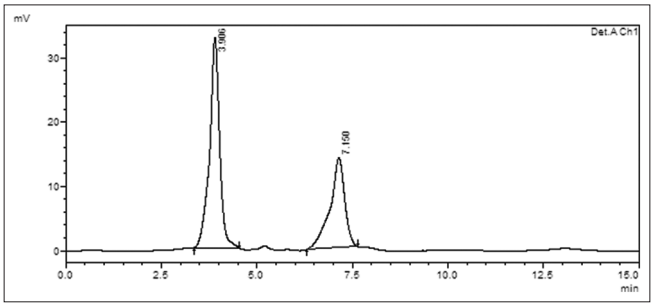

Figure 3: Force degradation-basic hydrolysis

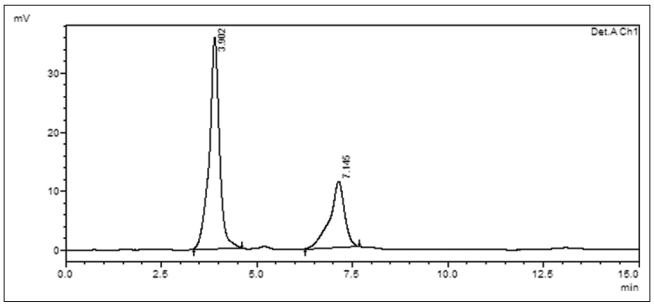

Figure 4: Force degradation-oxidative degradation

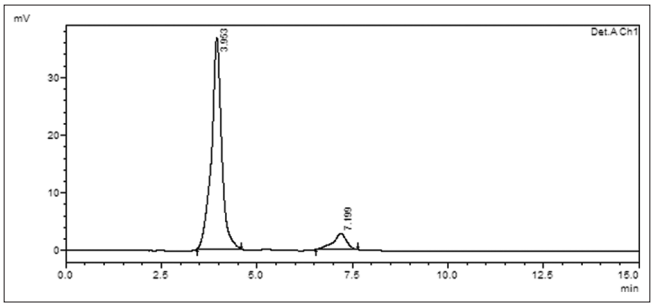

Figure 5: Force degradation-thermal degradation

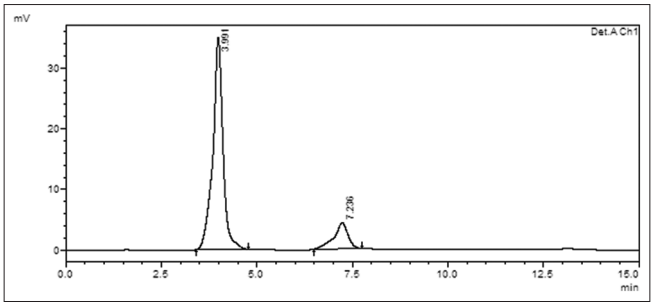

Figure 6: Force degradation-photolytic degradation (ultraviolet and visible light)

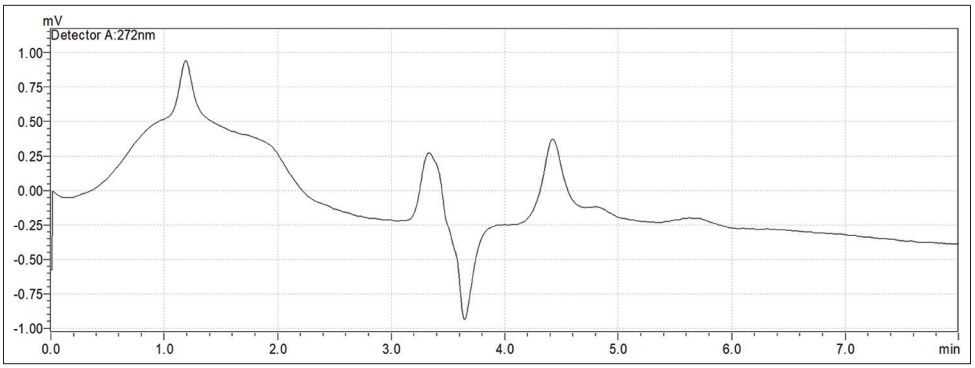

Figure 7: Analytical method validation active pharmaceutical ingredient-chromatogram

mobile phase composition, or detection wavelength; all parameters remain within accepted limits.

Accelerated stability studies of sublingual granules

The accelerated stability study results are presented in Table 4, showing the gradual degradation of rizatriptan benzoate over 6 months under stress conditions.

Chromatograms

The stability study demonstrated that rizatriptan benzoate granules remain stable under accelerated conditions, with drug content decreasing from 99.2% to 96.3% over 6 months. The slight color change observed at 6 months may indicate minor degradation, but remains within acceptable limits.

Statistical analysis

The method's dependability and adequacy for its intended use were validated by statistical analysis of the validation data. The relative standard deviation readings continuously fell below 2%, which satisfies the requirements for pharmaceutical analytical procedures to be validated. Excellent linearity between concentration and response was confirmed by the linearity study's correlation coefficient of 0.999, which was higher than the required minimum of 0.999.

Table 1: Linearity data of rizatriptan benzoate

Concentration (µg/mL)	Peak area
50	125,820
75	189,760
100	253,645
125	316,490
150	378,105

Table 2: Accuracy data of rizatriptan benzoate

Level (%)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
80	8.0	8.02	100.25
100	10.0	9.95	99.50
120	12.0	12.03	100.25

Table 3: Precision data of rizatriptan benzoate

Parameter	Mean (% assay)	RSD (%)
Intra-day precision	99.8	0.30
Inter-day precision	99.83	0.31

Table 4: Accelerated stability study results of formulation

Time point	Appearance	Assay (% drug content)	Moisture content (%)	pH
0 month	White granules	99.2	1.0	4.8
1 month	White granules	98.5	1.2	4.7
3 months	Off-white granules	97.6	1.4	4.6
6 months	Slightly yellow	96.3	1.6	4.5

DISCUSSION

A stability-indicating analytical method was developed to analyse a newly formulated multiparticulate drug delivery system of rizatriptan benzoate intended for sublingual administration. To establish the method's suitability, forced degradation studies were performed on the drug under hydrolytic, oxidative, thermal, and photolytic stress conditions. Rizatriptan benzoate was found to be susceptible to degradation, although the extent varied with the type of stress applied. Acidic conditions caused higher degradation than basic hydrolysis, likely due to protonation of the indole nitrogen or side-chain groups facilitating bond cleavage, while its stability in alkaline media indicated relative resistance to base-catalyzed hydrolysis. Oxidative stress generated distinct degradant profiles, consistent with the sensitivity of the tertiary amine and indole moieties to peroxide attack. Photolytic stress also caused degradation, though less pronounced, suggesting moderate photosensitivity requiring protective packaging. Thermal stress under dry heat produced

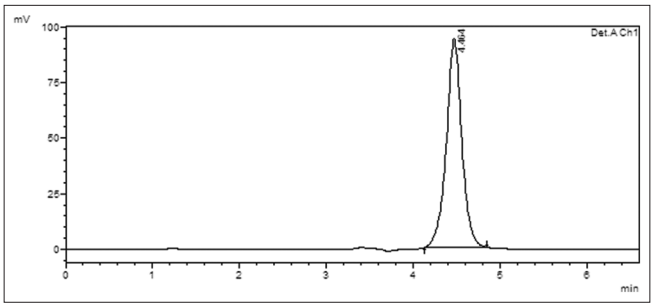

Figure 8: Analytical method validation of active pharmaceutical ingredient by low-quality control chromatogram

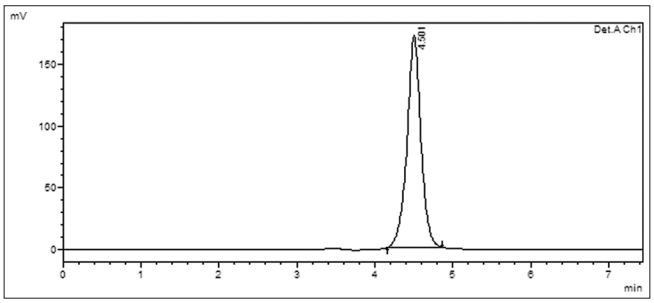

Figure 9: Analytical method validation active pharmaceutical ingredient by middle quality control-chromatogram

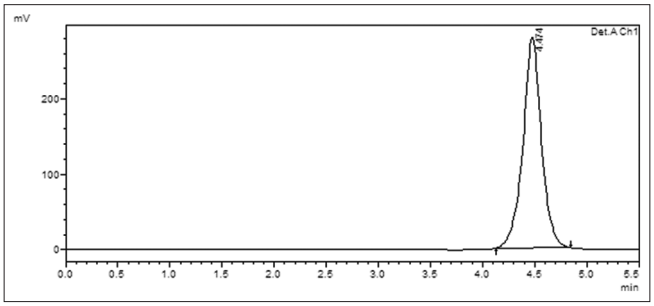

Figure 10: Analytical method validation active pharmaceutical ingredient by high-quality control-chromatogram

minimal impact, reflecting the compound's stability in the absence of reactive moisture. Since there was no evidence of drug excipient interactions therefore for degradation studies were not performed on rizatriptan benzoate granules.

The developed HPLC method for the drug successfully resolved rizatriptan benzoate from all degradation products with good resolution. A slight λmax shift to 227 nm was observed, possibly due to the presence of excipients and degradants. Since the formulation was under development at the time of analytical method development, the preliminary method development was carried out on the pure drug.

Validation studies on rizatriptan benzoate multiparticulate granules confirmed compliance with ICH guidelines for specificity, accuracy, precision, sensitivity, linearity, robustness, and ruggedness. Reported values included accuracy of 98.6%, precision (RSD <2%), and linearity (R^2 = 0.9996), establishing the method's reliability as a stability-indicating tool. Since the focus was on multiparticulate granules of rizatriptan benzoate to be administered sublingually the analytical method validation of pure drug was not carried out.

Accelerated stability studies of the multiparticulate formulation, stored at 40 ± 2°C and 75 ± 5% RH for 6 months in paper sachets, demonstrated only minor potency loss and a slight color change. The absence of significant changes supports the prediction that the formulation can remain stable for up to 24 months at room temperature. Observed anomalies, such as incomplete degradation under photolytic and thermal stress, likely reflect the structural stability of rizatriptan benzoate, while variations in peak intensities between stress conditions may be attributed to differences in degradation kinetics rather than analytical shortcomings. Therefore, the validated HPLC method is believed to be reliable, sensitive, and capable of detecting subtle degradation, making it suitable for routine stability monitoring of rizatriptan benzoate formulations. Since the long-term studies under the ICH guidelines[14-20] required a dedicated stability chamber for more than 2 years and were not available presently, therefore long-term studies could not be performed.

CONCLUSION

A stability-indicating analytical method was developed for a multiparticulate drug delivery system containing rizatriptan benzoate. As part of method development, extensive forced degradation investigations were carried out to successfully identify breakdown products of rizatriptan benzoate under a range of stress conditions. An HPLC method was successfully developed to identify and/or quantify the parent drug as well as the degradation product of rizatriptan benzoate. The HPLC method was used to propose the shelf-life of a multiparticulate drug delivery system containing rizatriptan benzoate stored at accelerated stability study conditions. The results obtained from the studies indicate that the developed and validated HPLC method can be used for stability testing, and pharmaceutical analysis of formulations containing rizatriptan benzoate. While accelerated studies suggest good shelf-life, confirmation through long-term real-time stability studies is suggested in future work. This research contributes to pharmaceutical analytical sciences by providing a comprehensive approach for multiarticulate drug delivery system evaluation, supporting regulatory compliance, and ensuring product quality throughout the pharmaceutical manufacturing process.

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