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Research Article

Development and Validation of a Stability-Indicating RP-HPLC Assay Method For The Estimation Of Rizatriptan Benzoate In Tablet Dosage Form

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ARTICLEINFO

ABSTRACT

This research endeavors to develop and validate a robust method for the assay of Rizatriptan Benzoate tablets utilizing RP-HPLC. Conducted at the Laboratory of ARL in Glenmark Research Centre, Navimumbai, the study encompasses comprehensive investigations into method development and validation protocols. The research begins with an introduction to method development and validation, elucidating various chromatography techniques, HPLC instrumentation, and analytical methodologies. A thorough literature survey reveals existing methods for the drug, laying the groundwork for subsequent experimentation. Subsequent chapters delve into the drug profile, including its physical characteristics, structure, solubility, pharmacological action, and ADME properties. The aim and objectives of the study are outlined, followed by a detailed exploration of the experimental procedures conducted during method development. Various approaches to HPLC method development are explored, with a focus on optimizing chromatographic conditions. The validation protocol is meticulously outlined, covering specificity, linearity, precision, accuracy, ruggedness, and robustness parameters. The validated method demonstrates exceptional performance characteristics, paving the way for routine assay applications in the pharmaceutical industry. Results of the assay method development, including optimized chromatographic conditions and standard concentrations, are presented.In conclusion, the validated method emerges as a reliable tool for the routine assay of Rizatriptan Benzoate tablets, offering specificity, precision, linearity, robustness, and sensitivity. This research contributes to the advancement of pharmaceutical quality control processes, ensuring the safety and efficacy of Rizatriptan Benzoate formulations.

Keywords: Rizatriptan Benzoate; RP-HPLC; Assay Method; Validation; Pharmaceutical Analysis

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1.Introduction

Rizatriptan Benzoate, chemically known as N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine benzoate, is a selective 5-hydroxytryptamine1 receptor subtype agonist. It is primarily used in the treatment of acute migraine attacks due to its potent vasoconstrictive properties on cranial blood vessels and inhibition of neuropeptide release. The therapeutic efficacy of Rizatriptan Benzoate relies heavily on accurate dosage administration, necessitating precise analytical methods for its quantification in pharmaceutical formulations [1].

As with any pharmaceutical compound, ensuring the stability of Rizatriptan Benzoate within its dosage form is paramount to guaranteeing its therapeutic efficacy and patient safety throughout the product's shelf-life. However, the inherent chemical instability of Rizatriptan Benzoate, particularly in the presence of environmental factors such as light, heat, and moisture, poses a significant challenge in pharmaceutical formulation and analysis [2].

Various analytical techniques have been employed to quantify Rizatriptan Benzoate in pharmaceutical formulations, including spectrophotometry, high-performance liquid chromatography (HPLC), and gas chromatography (GC). However, due to the compound's susceptibility to degradation, there is a critical need for a stability-indicating assay method that can accurately quantify Rizatriptan Benzoate while simultaneously detecting and quantifying any degradation products that may form under stress conditions [3].

In this study, we aim to address this gap by developing and validating a stability-indicating RP-HPLC assay method for the estimation of Rizatriptan Benzoate in tablet dosage form. This method will not only provide a reliable means of quantifying the active pharmaceutical ingredient (API) but also serve as a valuable tool for assessing the stability and quality of Rizatriptan Benzoate-containing pharmaceutical formulations [4].

Through systematic method development and validation, we seek to establish a robust analytical method that can withstand the challenges posed by Rizatriptan Benzoate's chemical instability, thereby ensuring the accurate determination of drug content and the integrity of pharmaceutical products throughout their shelf-life.

2. Materials and Method

2.1 Material

Rizatriptan benzoate, the active ingredient, was obtained from Glenmark Research Center, Mahepe, Navi Mumbai. We used analytical-grade acetonitrile and methanol sourced from Rankem Ltd., Mumbai, along with some standard laboratory reagents. Analytical equipment included an analytical balance (Mettler Toledo, Model no: AX205), ultrasonicator (Unichrome Associates, Model no: UCA701), and pH meter (Orion 3 Star). Chromatographic analyses were performed using Waters HPLC (Alliance Series 2695) and Shimadzu LC2010 (Class-VP series), while spectroscopic measurements utilized FTIR from Perkin Elmer and UV-Visible Spectrophotometry from the same supplier. Additional equipment included a melting point apparatus from Mettler Toledo [5].

2.2 Assay Method Development and Optimization

The assay method for Rizatriptan Benzoate underwent a meticulous development and optimization process, encompassing a series of

trials aimed at refining chromatographic parameters. In Trial 01, two replicates of a standard solution of Rizatriptan Benzoate (10µL each) were injected into the HPLC system employing a mobile phase comprising water:acetonitrile (50:50) on a Kromasil 100, C18 column at a flow rate of 1.5 mL/min, with detection set at 228 nm and an initial isocratic elution mode. Subsequently, Trial 02 introduced a variation in the mobile phase composition to water:acetonitrile (60:40) while maintaining the isocratic elution mode. Trial 03 transitioned to a gradient elution mode, employing a mobile phase consisting of buffer:acetonitrile on the same column at a flow rate of 1.5 mL/min, with the gradient program detailed in Table 6.4. Building upon Trial 03, Trial 04 utilized a modified gradient program (detailed in Table 6.5) with a reduced flow rate of 1.0 mL/min to further optimize the assay method [6].

2.3 Assay Method Development, Optimization, and Validation

The HPLC method for determining the assay of Rizatriptan Benzoate in Rizatriptan Benzoate tablets (10mg) underwent rigorous development, optimization, and validation steps [7-10].

Mobile Phase Preparation:

Mobile phase A was prepared by dissolving 0.1% triethylamine (TEA) in 1000mL of water and adjusting the pH to 2.5 with orthophosphoric acid (OPA). Mobile phase B consisted of acetonitrile [11].

Preparation of Diluent:

Orthophosphoric acid (1.0 mL) was diluted in 1000 mL of water to prepare the diluent.

Chromatographic Conditions:

A mobile phase comprising buffer:acetonitrile (gradient mode) was used with a Kromasil 100, C18 column (150 x 4.6 mm, 5µ). The flow rate was maintained at 1.0 mL/min, with detection at 228 nm. The column temperature was set at 50°C, and the injection volume was 10µL [12-14].

Standard Solution Preparation:

Rizatriptan Benzoate standard solution was prepared by accurately weighing 72.7 mg (equivalent to 50 mg of Rizatriptan) and diluting it in a 100 mL volumetric flask with diluent. Further dilution was done to obtain a standard concentration of 25 ppm.

Sample Solution Preparation (For 10 mg Tablet):

Ten tablets were powdered and transferred into a 200 mL volumetric flask, followed by the addition of 150 mL of diluent. After sonication, cooling, and filtration, the solution was further diluted to obtain a sample preparation of 25 ppm [15].

Placebo Solution Preparation:

Equivalent to 50 mg of placebo, accurately weighed placebo was diluted in a 100 mL volumetric flask with diluent. After sonication, centrifugation, and filtration, the solution was further diluted to obtain a placebo preparation of 25 ppm [16].

Blank Preparation:

Diluent was used as the blank solution.

System Suitability Evaluation:

System suitability was assessed by injecting the standard solution five times into the HPLC. The area counts of Rizatriptan Benzoate peaks were measured, ensuring the relative standard deviation did not exceed 2.0%. Additionally, the tailing factor for the Rizatriptan peak was ensured to be less than 2.0, with a minimum of 3000 theoretical plates [17].

Calculation:

The percentage of Rizatriptan Benzoate was calculated using the provided formula, considering the average area count of Rizatriptan Benzoate peaks in both sample and standard solutions, the percent potency of the Rizatriptan Benzoate

working standard, label claim, and molecular weights.

2.4 Validation Parameters

Specificity

Preparation of standard test solution involved accurately weighing and transferring rizatriptan benzoate working standard into a volumetric flask, followed by dilution and sonication. Sample and placebo solutions were prepared similarly, with appropriate dilutions and filtration steps. Spike solutions were prepared by adding impurity solution to the sample solution [18-20].

Linearity

A series of standard preparations covering a range of concentrations were prepared to establish linearity. These solutions were diluted to various concentrations, and their correlation coefficients were assessed to ensure linearity within the desired range [21].

Accuracy (Recovery)

Placebo solutions were spiked with Rizatriptan Benzoate at different levels, and triplicate analyses were performed. The recovery rates were calculated to assess the accuracy of the analytical method [22-23].

Precision

System precision involved injecting replicate standard solutions to assess instrument precision. Method precision was evaluated by analyzing multiple sample preparations independently prepared by one analyst. Intermediate precision was determined by analyzing standard and sample solutions using different HPLC systems, analysts, and columns [24].

Robustness

The robustness of the method was evaluated by introducing variations in chromatographic conditions, such as pH of buffer, flow rate, column temperature, and wavelength. The system suitability was assessed under these variable conditions to ensure the method's robustness.

Stability of Analytical Solution

The stability of analytical solutions was determined by comparing the assay of old standard solutions against freshly prepared ones over a specified duration to ensure that the solutions remained stable over time [25].

3. Result and Discission

3.1 Assay Method Development

The assay method for Rizatriptan underwent several trial runs to optimize its chromatographic conditions. Here, we discuss the results of each trial and the subsequent modifications made to achieve an accurate and reliable analysis.

Trial 1:

In the initial trial, the chromatogram revealed that the Rizatriptan peak was retained earlier with fronting observed. This indicated a need for modification in the method to improve peak resolution and shape.

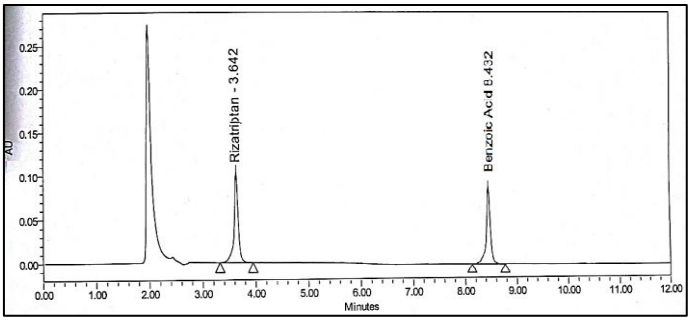

Figure 1: Typical chromatogram of trial 1

Trial 2:

In the second trial, similar issues were encountered, with the Rizatriptan peak still being retained earlier and exhibiting fronting. As a result, the method was altered to gradient mode to address these concerns.

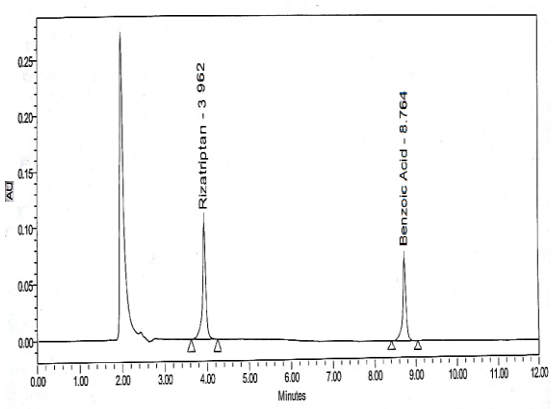

Figure 2: Typical chromatogram of trial 2

Trial 3:

Despite the modification to gradient mode, trial 3 demonstrated that the Rizatriptan peak continued to be retained earlier. However, the resolution between Rizatriptan and benzoic acid was improved. Yet, tailing was observed in both peaks, indicating the need for further adjustments.

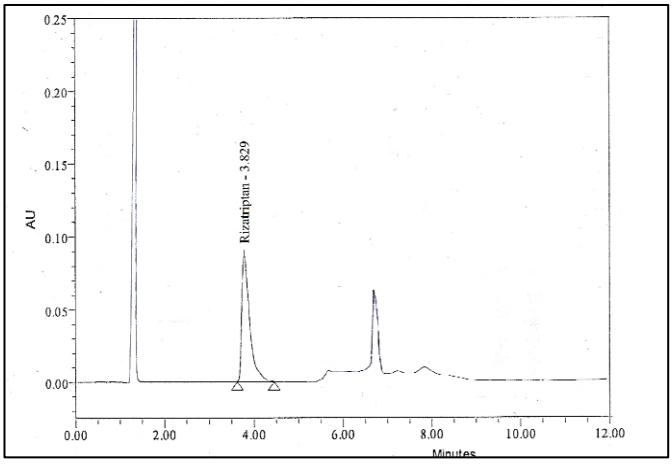

Figure 3: Typical chromatogram of trial 3

Trial 4:

The fourth trial showcased promising results. While the Rizatriptan peak was retained at 5.340 minutes, the peak shape was sharp and passed the peak purity test. Additionally, the resolution between Rizatriptan and benzoic acid was satisfactory, with a tailing factor of 1.27, and a resolution of >2. Consequently, these optimized chromatographic conditions were deemed suitable for the assay of Rizatriptan.

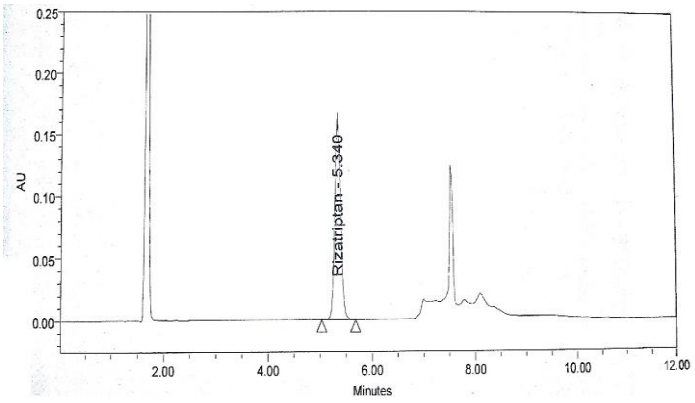

Figure 4: Typical chromatogram of trial 4

3.4 Assay Method Validation

3.4.1 Specificity

Identification:

The retention time of the rizatriptan benzoate peak in the chromatogram of the sample preparation corresponds to that of the rizatriptan peak in the chromatogram of the standard preparation, respectively. Specifically, the retention time of the rizatriptan peak in the standard solution is 5.340 minutes, while the retention time of the rizatriptan benzoate peak in the sample solution is 5.334 minutes (Table 1).

Table 1: Table for retention time (identification)

Sample information.	Retention time (minutes)
Rizatriptan Benzoate standard	5.340
Rizatriptan Benzoate sample	5.334

Placebo Interference:

Representative placebo solutions, standard solutions, and sample solutions of Rizatriptan Benzoate OD Tablet were prepared and analyzed. No interference was observed from blank and placebo solutions at the retention time of the rizatriptan peak. Both the standard and sample solutions exhibited purity, as indicated by the purity angle and threshold values (Table 2).

Table 2: Table for Specificity

Sr. No.	Name	Purity Angle	Purity Threshold
1	Standard solution – rizatriptan	0.104	1.104
2	Sample solution -rizatriptan benzoate	0.103	1.103

Known Impurity Interference:

Three samples of rizatriptan benzoate orally disintegrating tablet were analyzed by spiking with known related substances at a 1% level. The assay results were compared with un-spiked samples. The % assay mean for unspiked and spiked samples were found to be 98.4% and 97.6% respectively, with a difference of less than 1%. Additionally, the peak purity data indicated no impurity interference, affirming the specificity of the method (Table 3).

Table 3: Table for known impurity interference for rizatriptan

	Control Sample (% label claim)	**Spiked sample (% label claim)**
1	99.0	96.7
2	97.9	98.1
3	98.2	98.0
Mean	**98.4**	**97.6**
SD	**0.569**	**0.781**
RSD	**0.58**	**0.80**

Forced Degradation Studies:

Forced degradation studies were conducted under various conditions. The results showed In figure 5 that the rizatriptan peak remained homogeneous with well-resolved peaks in each degradation sample, meeting the acceptance criteria. This indicates that the method is stability indicating and specific.

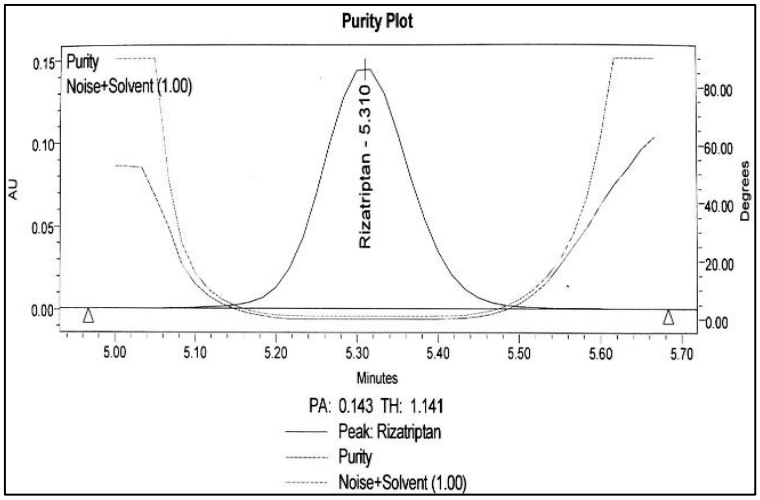

Figure 5: Purity plot of rizatriptan in Thermal degradation

3.4.2 Linearity and Range

For establishing the linearity for rizatriptan benzoate, a series of a standard preparation of rizatriptan benzoate were prepared to cover a range of 50 % to 150 % of sample concentration i.e. 25ppm of rizatriptan in rizatriptan benzoate Orally disintegrating Tablets. (Minimum 5 points in the range 80-120% of standard/sample concentration for assay) based on this the range proposed for Linearity determination is 50% to 150% test concentration (i.e. 12.5 ppm to 37.5 ppm) for rizatriptan.

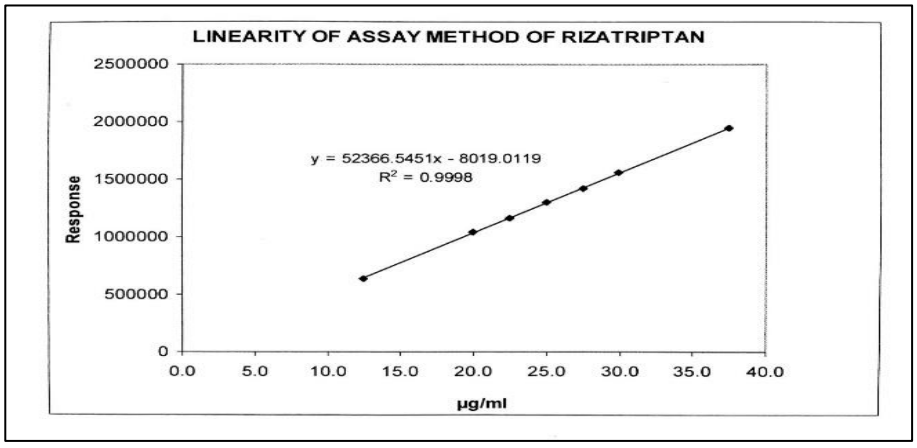

Figure 6: Linearity assay graph of rizatriptan benzoate

3.4.3 Accuracy (Recovery)

Placebo of rizatriptan was spiked with rizatriptan drug substance at three different levels: 80%, 100% and 120% of the label claim in triplicate (in total nine determinations) and then proceeded with sample solutions as describe under methodology. Each of the sample solution was injected in duplicate and the average area count was taken for calculation.

Mean recovery should be in the range of 98.0% to 102.0%. The RSD should not be more than 2.0%. Mean recovery for rizatriptan benzoate is 100.7% and RSD is 0.25%.. Therefore, the HPLC method for the determination of rizatriptan in rizatriptan benzoate orally disintegrating tablet is accurate.

3.4.4 Precision

System Precision:

Five replicate injections of the Standard preparation were injected into the HPLC system using the method as described under Methodology. RSD should not be more than 2.0%. The RSD of system precision is 0.88%. Therefore, the HPLC method for the determination of Assay of rizatriptan in rizatriptan benzoate orally disintegrating tablets has been precise.

Table 6: Data sheet for System Precision.

Injection	Area
1	1294249
2	1301446
3	1306403
4	1311563
5	1324722
Mean	**1307687**
SD	**11490.204**
%RSD	**0.88**

3.4.5 Ruggedness (Intermediate precision)

The standard solutions & six sample solutions of rizatriptan benzoate orally disintegrating tablets (10mg) of the same lot using a different HPLC system, different analyst, a different column on a different day will be analyzed. The mean and percent RSD values for the area will be calculated. Overall RSD for twelve results should not be more than 2.0% The RSD of Ruggedness (intermediate precision) is 1.03% for rizatriptan. Therefore, the HPLC method for the determination of Assay of rizatriptan in rizatriptan benzoate orally disintegrating tablet has been rugged.

3.4.6 Robustness

Three sample preparations of the same lot of rizatriptan benzoate orally disintegrating tablets were prepared. The sample along with standard preparations was injected in duplicate under the different chromatographic condition as shown below.

Table 8: Table for Change in pH Buffer (± 0.2 units):

Control	(+ 0.2)	(-0.2)
98.3	98.3	98.1
99.7	97.7	98.7
98.0	98.1	97.9
Cumulative Mean	**98.4**	**98.5**
Cumulative SD	**0.698**	**0.675**
Cumulative %RSD	**0.71**	**0.69**

Table 4: Table for Linearity

% Concentration	Concentration (µg per mL)	Response (Area)	Statistical analysis	
50%	12.46	639537	**Slope**	**52366.5**
80%	19.93	1039370		
90%	22.42	1166985	**Intercept**	**-8019.0**
100%	24.91	1301725		
110%	27.40	1419353	**Correlation Coefficient**	**0.9998**
120%	29.89	1563393		
150%	37.37	1944822		

Table 5: Table for Accuracy

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 80% -1	80.85	81.43	**100.7**
Accuracy 80% -2	80.38	81.09	**100.9**
Accuracy 80% -3	79.82	80.43	**100.8**
Accuracy 100% -1	99.58	100.14	**100.6**
Accuracy 100% -2	99.93	100.74	**100.8**
Accuracy 100% -3	100.26	100.34	**100.1**
Accuracy 120% -1	119.38	120.06	**100.6**
Accuracy 120% -2	120.10	121.18	**100.9**
Accuracy 120% -3	120.30	120.87	**100.5**
Mean			**100.7**
SD			**0.251**
% RSD			**0.25**

Table 7: Table for Ruggedness

Sample	Analyst 1 % Assay	Analyst 2 % Assay
1	99.4	98.3
2	98.2	99.7
3	99.0	98.0
4	99.5	998.4
5	96.7	96.6
6	97.6	98.0
Mean	**98.4**	**98.2**

SD	**1.108**	**0.993**
% RSD	**1.13**	**1.01**
Overall Mean	**98.3**	
Overall SD	**1.011**	
Overall % RSD	**1.03**	

Conclusion

In conclusion, this research conducted at the Laboratory of ARL in Glenmark Research Centre, Navimumbai, focused on the method development and validation of the Assay for Rizatriptan Benzoate tablet by RP-HPLC. Beginning with an introduction to method development and validation, the study explored various chromatography techniques, HPLC instrumentation, and analytical methodologies. A comprehensive review of existing methods for the drug was presented in the literature survey.

The drug profile, encompassing its physical appearance, structure, solubility, pharmacological action, and ADME properties, was detailed in the third chapter. The aim and objectives of the study were outlined in the fourth chapter, followed by a delineation of the experimental procedures conducted during method development in the fifth and sixth chapters.

The seventh chapter discussed different approaches in HPLC method development, detailing the procedures for optimizing chromatographic conditions. The eighth chapter covered the protocol for method validation, while the ninth chapter compiled the validation data sheet. The validated method was found to be specific, linear, precise, accurate, rugged, and robust for the assay of Rizatriptan Benzoate in its solid dosage form.

The results of assay method development revealed the optimized chromatographic conditions, including the column, mobile phase composition, flow rate, detection wavelength, pump mode, column temperature, and injection volume. The standard concentration used for the assay was 25 ppm Rizatriptan Benzoate.

In summary, the final validated method demonstrated specificity, precision, linearity, robustness, and sensitivity, as confirmed through comprehensive method validation. Thus, this method can be reliably implemented for the routine assay of Rizatriptan Benzoate in Rizatriptan Benzoate Tablets, contributing to the pharmaceutical industry's quality control processes.

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