Original Article

Rp-Hplc Method For The Estimation Of Rizatriptan Benzoate Tablets

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Abstract

A simple reverse phase HPLC method was developed for the simultaneous determination of rizatriptan benzoate, in pharmaceutical dosage form. An Inertsil C_8 (150 × 4.6 mm), 5µ column from Schimadzu in gradient mode, with mobile phases pH 3.0 Potassium dihydrogen phosphate buffer and methonal was used. The flow rate was 2.0 ml/min and effluent was monitored at 225 nm. The retention time was 6.21 min. As per ICH guide lines the method was validated over the range of 25–150 µg/mL for the analyte, and is accurate (average accuracies of three different concentrations ranged from 98.3 to 99.1% for rizatriptan Benzoate. The proposed method can be used as alternative method to the reported ones for the routine determination of selected drugs under the study in pharmaceutical dosage forms.

Keywords: Rizatriptan benzoate, Dosage form, tablets, HPLC, Phosphate buffer.

INTRODUCTION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage forms is more important in pharmacokinetic, toxicological and biological studies. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry is under increased scrutiny from the government and the public interested groups to contain costs and at consistently deliver to market safe, efficacious product that fulfill unmet medical needs.[1]

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.[2],[3]

Rizatriptan is a 5-HT receptor agonist that has weak affinity for other 5-HT, receptor subtypes and the 5-HT, receptor, but no significant activity at other 5-Hreceptor or at alpha or betaadrenergic,[4] dopaminergic, histaminergic muscarinic, or benzodiazepine receptors.The presumed mechanism of actionof rizatriptan is through activation of postsynaptic 5-HT,, receptors within cerebral and dural vessel walls, causing vasoconstriction and inhibition of trigeminaperivascular nerve terminals. In addition, activation of presynaptic 5-HT, receptors by rizatriptan prevents release of vasoactive neuropeptides and blocksdepolarization of trigeminal axons. Rizatriptan is also believed to act centrallyin the brain stem, thereby blocking the transmission of pain.

An HPLC method for rizatriptan in rata plasma was developed using C18 (250 x 4.6 mm, 5µ) column using 10 mM di-potassium hydrogen orthophosphate buffer (pH 3.2) and methanol in the ratio of 77:23.[5] In another study

a RP-HPLC method was developed for estimation of rizatriptan benzoate in oral strip formulations.[6] Force degradation study of rizatriptan benzoate by RP- HPLC method was performed.[7]

A survey of literature reveals that few analytical methods are available for the drugs like rizatriptan benzoate.[8] The existing phyisico-chemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for estimation rizatriptan benzoate in pharmaceutical dosage forms adopting different available analytical techniques like HPLC. According to the literature survey it was found that few analytical methods on HPLC-MS/MS, HPLC were reported for rizatriptan benzoate.

The objective of the proposed methods is to develop simple and accurate methods for the estimation of rizatriptan benzoate in pharmaceutical dosage forms by HPLC. Method development by RP-HPLC method for rizatriptan benzoate involes the development of suitable mobile phase, optimization of the chromatographic conditions, selection of suitable detection wavelength, preparation of standard calibration curve of rizatriptan benzoate, assay of pure mixed standards and formulation and finally validation of the developed method.

MATERIALS AND METHODS

Rizatriptan benzoate was gift sample from Nishka Labs, Hyderabad, India. HPLC grade Methanol and triethylamine were obtained from Merck India, Mumbai. Milli Q grade water was employed. AR grade Potassium dihydrogen orthophosphate and orthophosphoric acid were purchased from Loba Chemie, Mumbai, India.

Preparation of pH 3.0 buffer solution

Weighed and dissolved accurately about 2.7 gm of potassium dihydrogen orthophosphate in 100 ml of water. Added 2 ml of triethylamine and adjusted the pH to 3.0±0.05 with orthophosphoric acid. Filtered the solution through 0.45 µm nylon membrane filter.

Preparation of mobile phase

A gradient mixture of pH 3.0 buffer solution and methanol were used in the ratio of 80:20 (v/v).

Preparation of diluent

Prepared a degassed mixture of buffer and methanol in the ratio of 80:20 (v/v).

Chromatographic conditions[9]

Column : Inertsil C_8, 150 x 4.6mm or 5µm equivalent

Flow rate : 2.0 ml/min

Wave length : UV, 225 nm

Injection volume : 10 µl

Column température : 40 ºC

Run time : 17 min

Gradient programme

Time (min)	Buffer pH 3.0 (%v/v)	Methanol (%v/v)
$T_{0.01}$	90	10
T_8	90	10
T_9	30	70
T_{11}	30	70
T_{12}	90	10
T_{17}	90	10

Preparation of solutions

Standard solution

Weigh and transfer accurately about 72 mg of rizatriptan benzoate working standard into a 100 ml clean, dry volumetric flask, add about 50 ml of diluent, sonicate to dissolve dilute to volume with diluent and mix. Dilute 5 ml of the solution to 59 ml with diluent and mix. Filter the solution through 0.45µm nylon membrane filter.

Sample solution (5 mg strength)

Weigh and transfer 10 tablets into a 100 ml, clean, dry volumetric flask add about 50 ml of diluent, sonicate for 20 min with intermittent shaking, dilute to volume with diluent and mix. filter the solution through 0.45µm nylon membrane filter.

Sample solution (10 mg strength)

Weigh and transfer 10tablets into a 200 ml, clean, dry volumetric flask add about 100 ml of diluent, sonicate for 20 min with intermittent shaking, dilute to volume with diluent and mix. Filter the solution through 0.45µm nylon membrane filter.

Evaluation of system suitability

Inject the standard solution in five replicate injections into the chromatograph and record the chromatograms. The column efficiency as determined from rizatriptan peak is not less than 2000 USP plate count and the tailing factor for the same peak is not more than The column efficiency as determined from rizatriptan peak is not less than 2.0. The %RSD for the peak areas of the five replicate injections from standard is not more than 2.0. Inject the sample solution, in duplicate into the chromatograph. Record the chromatograms and measure the peak areas. Retention time of rizatriptan is about 5.2 min.

Calculation

The amount of rizatriptan ($C_{15}H_{19}N_5$) is calculated by using the formula.

$$\text{Content of rizatriptan} = \frac{A_T \times D_S \times P \times 269.35}{A_S \times D_S \times 100 \times 391.47}$$

Where,

$A_{T==}$average area count of rizatriptan peak in the chromatograms of sample solution

$A_{S=}$ average area count of rizatriptan peak in the chromatograms of standard solution as obtained under system suitability.

$D_{S\,=}$ Dilution factor of standard solution

$D_{T\,=}$ Dilution factor of sample solution.

P = Percentage purity of rizatriptan benzoate working standard used (as is basis)

Molecular weight of rizatriptan = 269.35.

Molecular weight of rizatriptan benzoate =391.47.

Method validation [10]

1. System suitability and system precision

A Standard solution was prepared by using rizatriptan, working standards as per test method and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for rizatriptan retention times and peak areas.

Acceptance criteria

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %

2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.

3. The number of theoretical plates (N) for the rizatriptan peaks is NLT 2000.

4. The Tailing factor (T) for the rizatriptan peaks is NMT 2.0.

2. Specificity (Interference from degradation products)

A study was conducted to demonstrate the effective separation of degradants from rizatriptan. Separate portions of Drug product exposed to following stress conditions to induce degradation.[11]

a) Water degradation

b) Acid degradation

c) Base degradation

d) Peroxide degradation

e) Thermal degradation

f) UV degradation

g) Humidity degradation

Stressed samples were injected into the HPLC system with photo diode array detector by following test method conditions. All degradant peaks were resolved from, peaks in the chromatograms of all samples and placebo did not shown any considerable peaks under the above conditions. The chromatograms of stressed samples were evaluated for peak purity of rizatriptan, using water's Empower software. For all forced degradation samples the degradants should not interference in quantitating the rizatriptan.[12]

Acceptance criteria: Purity angle should be less than Purity Threshold. Rizatriptan and its degraded substances should not have any flag in purity results table.

3. Precision[13]

a) System precision: Standard solution prepared as per test method and injected five times.

b) Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Acceptance criteria: The % relative standard deviation of individual rizatriptan, from the six units should be not more than 2.0%. The assay of rizatriptan, should be not less than 95.0% and not more than 105.0%.

4. Accuracy (recovery) [14]

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of rizatriptan, into each volumetric flask for each spike level to get the concentration of rizatriptan, equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of rizatriptan was calculated. Separately inject the blank, placebo, rizatriptan in to the chromatograph.

Acceptance criteria: The mean % recovery of the rizatriptan, at each level should be not less than 95.0% and not more than 105.0%.

5. Linearity of test method

A Series of solutions are prepared using rizatriptan working standard at concentration levels from 50% to 150% of target concentration (10%, 25%, 50%, 100%, 125% and 150%).Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance criteria: Correlation Coefficient should be not less than 0.9990. % of y- Intercept should be ±2.0. % of RSD for level 1 and Level 6 should be not more than 2.0%.

6. Ruggedness of test method

i**) System to system/analyst to analyst/column to column variability:** System to system/Analyst to Analyst/column to Column variability study was conducted on different HPLC systems, different columns and different analysts under similar conditions at different times. Six samples were prepared and each were analysed as per test method.

The relative standard deviation for rizatriptan, were found to be below 2 % on the columns, systems and Analysts.

Comparison of both the results obtained on two different HPLC systems, different column and different analysts shows that the assay test method is rugged for System to system/Analyst to Analyst/column to Column variability.

Acceptance criteria: The % relative standard deviation of rizatriptan, from the six sample preparations should be not more than 2.0%. The % of rizatriptan, should be between 95.0%-105.0%.

ii) Bench top stability of standard and test preparation: A study to establish stability of rizatriptan standard and test preparations on bench top was conducted over period of two days. Rizatriptan test preparation spiked to target concentration are injected initial 4.0Hr, 8 Hr, 12Hr, 16Hr, 23Hr and 28Hr. The difference in % of rizatriptan from initial to 28 h is within the limits. In the similar way standard preparations were injected initial 4.0Hr, 8 Hr, 12, 16, 23 and 28 h. From the above study, it was established that the standard and test preparations were stable for a period of 28 h on bench top.

Acceptance criteria: The difference between initial and bench top stability sample for % of rizatriptan should be not more than 3.0. The % assay of standard kept on bench top should not differ from initial value by more than 2.0.

Rrobustness

i) Effect of variation in mobile phase composition: A study was conducted to determine the effect of variation in Organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into the HPLC system using two mobile phases. The system suitability parameters were evaluated and found to be within the limits for mobile phase having 95% and 110% of method highest organic phase. Rizatriptan blend solution at target concentration was chromatographed using mobile phase having 95% and 110% of the method organic phase.

Aacceptance criteria: The tailing factor of rizatriptan, standards should be NMT 2.0 for Variation in Organic Phase.

ii) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.9ml/min and 1.1ml/min flow. Rizatriptan was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min. From the above study it was established that the allowable variation in flow rates is 0.9ml/min and 1.1ml/min.

Acceptance criteria: The tailing factor of rizatriptan, standards should be NMT 2.0 for Variation in Flow.

iii) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 30ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 30ºC. Similarly sample solution was chromatographed at 30ºC temperature. Rizatriptan was resolved from all other peaks and the retention times were comparable with those

Acceptance criteria: The tailing factor of rizatriptan, standard and sample solutions should be NMT 2.0 for Variation in temperature.

iv) Effect of variation of pH:

A study was conducted to determine the effect of variation in pH. Standard and sample solutions were prepared as per the test method and injected into the HPLC system using pH 2.4 and 2.8. The system suitability parameters were evaluated and found to be within the limits for pH 2.4 and 2.8. Rizatriptan was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having pH 2.5. From the above study it was established that the allowable variation in pH 2.4 and 2.8.

Aacceptance criteria: The tailing factor of rizatriptan, standard should be NMT 2.0 for Variation in pH.

RESULTS AND DISCUSSION

Linearity of rizatriptan was found between 12-75 µg/ml with an r^2 value of 0.999. System suitability and system precision showed %RSD for retention times and peak areas were found to be within the limits as shown in **Table 1**. Specificity is measured as purity angle which was found to be less than threshold angle in all forced degradation studies without having signs of purity flags. Thus, this method is considered to be "Stability Indicating". Refer **Table 2.** Test results are showing that the test method is precise as referred in **Table 3** for system precision and 4 for method precision. The recovery results indicating that the test method has an acceptable level of accuracy as shown in **Table 5**. Linearity of the developed method was found as follows. The correlation coefficient was found to be 0.9998 (**Table 6**). From the above study it was established that the linearity of test method is from 10% to 150% of the target concentration. For linearity plots see **Figure 3**. Ruggedness was found as the % relative standard deviation of rizatriptan, from the six sample preparations should be not more than 2.0%. The % of rizatriptan, should be between 95.0%-105.0% and it was found to be within the limits. Bench top stability of standard and test preparation % assay of standard kept on bench top should not differ from initial value by more than 2.0 and the % assay was found within the limits. Rizatriptan, was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having 100% of the organic phase.

From the study it was established that the allowable variation in mobile phase composition is 95% to 110% of the method highest organic phase of mobile phase. The tailing factor for rizatriptan, are found to be within the limits. Hence the method was robust. The Tailing Factor of rizatriptan, standards should be NMT 2.0 for Variation in Flow. The tailing factor for are found to be within the limits. During the test of the effect of variation in temperature, the tailing factor for rizatriptan, are found to be within the limits. From the results of the effect of variation of pH, the tailing factor for rizatriptan, are found to be within the limits (**Table 7**). From the above results it is concluded that the method is robust.

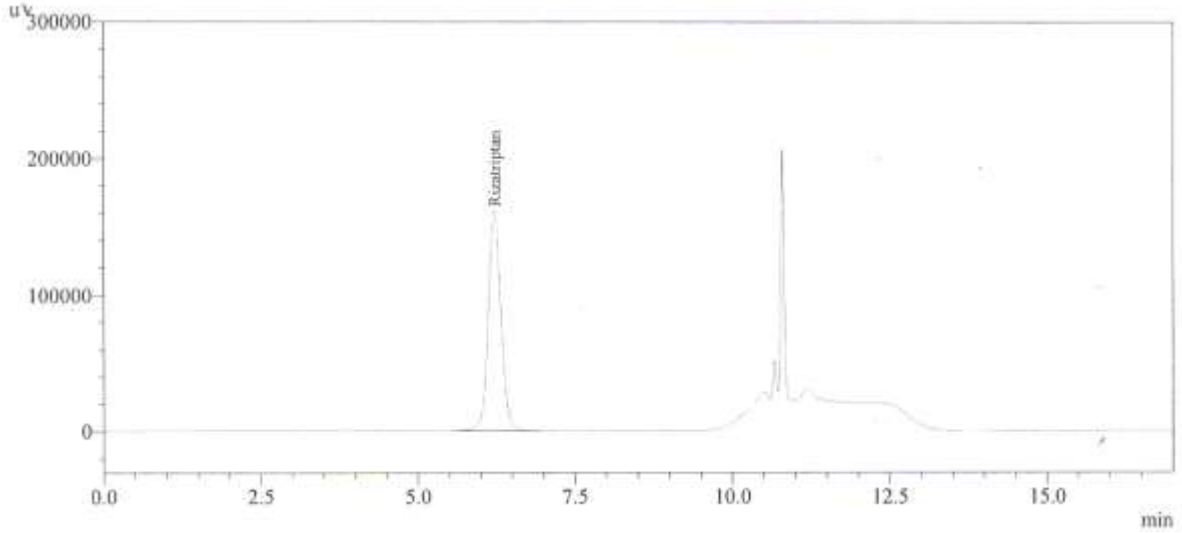

Fig 1: A typical chromatogram of rizatriptan benzoate standard

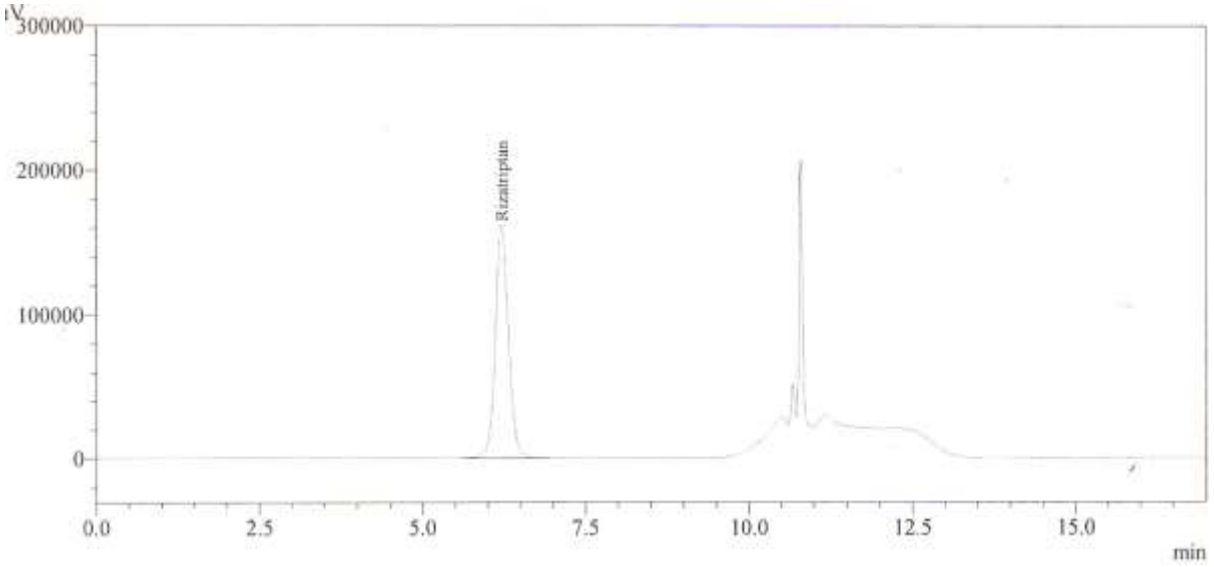

Fig 2: A typical chromatogram of rizatriptan benzoate sample

Table 1: System suitability and system precision

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	6.44	2263476	4471	0.9
2	6.41	2263518	4486	0.9
3	6.42	2263244	4423	1.0
4	6.40	2263371	4438	1.1
5	6.44	2263876	4412	1.0
6	6.39	2263424	4321	0.9
7	6.35	2263546	4465	1.1
8	6.46	2263478	4378	1.0
9	6.43	2263564	4398	1.2
10	6.42	2263543	4321	0.9
Mean	6.41	2263434	4331	0.99
SD	0.0309	172.388	---	---
% RSD	0.004	0.000076	---	---

Table 2: Interference from the degradation products

Degradation mechanism/condition	Observation
Protected sample	No interference at RT of analyte peak
Water/Reflux – 30.0 min	No interference at RT of analyte peak
Acid degradation 0.1 N HCl Reflux – 30.0 min	No interference at RT of analyte peak
Base degradation 0.01 N NaOH Reflux 30.0min	No interference at RT of analyte peak
Peroxide degradation 3.0% H_2O_2 Reflux – 30.0min	No interference at RT of analyte peak
Thermal degradation At 105°C - 48 h	No interference at RT of analyte peak
Photolytic degradation At 254nm - 24 h	No interference at RT of analyte peak
Accelerated degradation At 40°C/75% RH - 168 h	No interference at RT of analyte peak

Table 3: System precision

Concentration	Injection	Peak Areas of Rizatriptan
100%	1	2236212
	2	2236754
	3	2236543
	4	2236576
	5	2236231
Statistical **Analysis**	**Mean**	2236463
	SD	234.8802
	% RSD	1.050

Table 4: Method precision of rizatriptan

Tablet ID	% Assay		Statistical Analysis
1	98.5	Mean	99.3
2	100.2		
3	99.3	SD	0.7
4	99.4		
5	98.6	%RSD	0.765
6	100.3		

Table 5: Accuracy data of rizatriptan

Concentration % of spiked level	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	36.9	36.5	98.9	MEAN	99.1
50% Sample 2	37.0	36.8	99.5	SD	0.3464
50% Sample 3	37.1	36.6	98.9	% RSD	0.0034
100% Sample 1	71.6	70.6	98.6	MEAN	98.3
100% Sample 2	71.8	70.4	98.1	SD	0.264
100% Sample 3	71.8	71.2	98.2	% RSD	0.0026
150% Sample 1	107.7	106.2	98.7	MEAN	98.86
150% Sample 2	107.6	106.3	98.8	SD	0.2081
150% Sample 3	107.5	106.4	99.1	% RSD	0.0021

Table 6: Linearity of rizatriptan

Linearity Level	Concentration (µg/ml)	Average Area	Statistical Analysis	
L1-25%	12.52	691171	Slope	54026
L2-50%	25.05	1376566	y-Intercept	-1.05941
L3-75%	37.57	2115483	% of y- Intercept	-0.0000383
L4-100%	50.10	2765823	Correlation Coefficient	0.99998
L5-125%	62.62	3382156	r^2	0.9995
L6-150%	75.15	4072579		

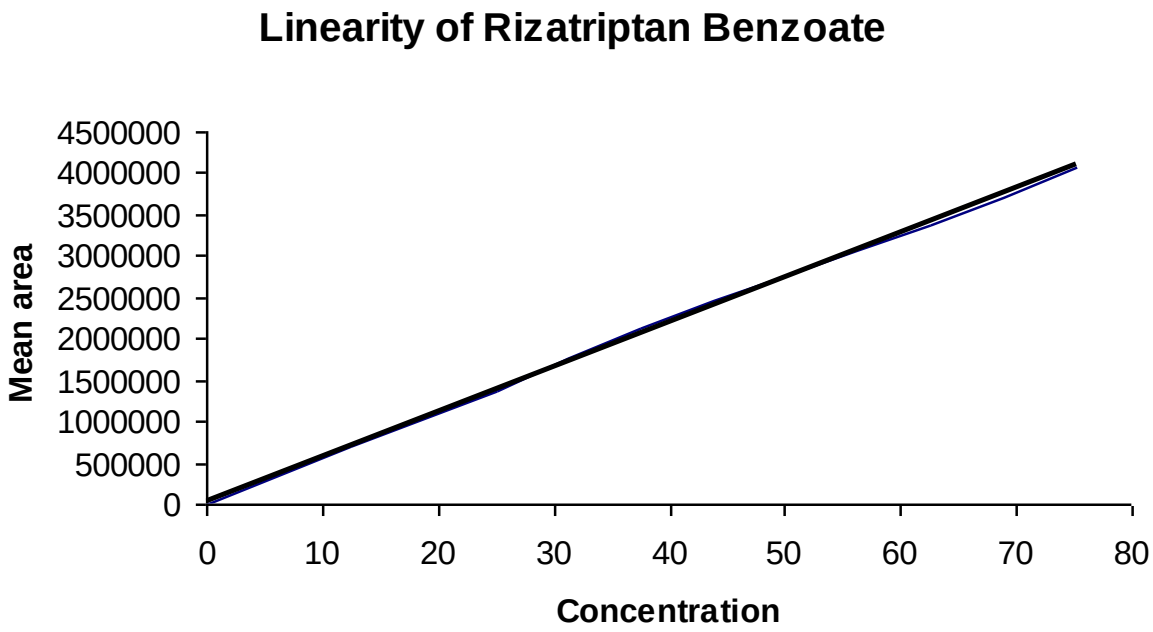

Figure 3: Linearity plot of rizatriptan

Table 7: Robustness of the method

Parameters	Optimum range	Conditions in procedure	Remarks
Mobile phase composition (% Of Acetonitrile)	10% variations in gradient conditions	Gradient	Beyond the optimum range of % of Acetonitrile, the resolution factor and relative retention and asymmetry factor were decreased
Flow rate ml/min	0.9-1.1	1.0	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was decreased
Temperature	25-30ºC	Ambient	Beyond the optimum range peak shape and symmetry was lost
PH of mobile phase	2.5-2.6	2.5	Beyond the optimum range of pH of the mobile phase, better resolution was not found. When it is reduced or increased beyond optimum range asymmetry factor was increased.

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