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Development and validation of High Performance Liquid Chromatographic method for estimation of Rizatriptan in dosage forms

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ABSTRACT

A new RP-HPLC method was developed for the estimation of Rizatriptan in tablets and it was validated as per ICH guidelines. The chromatogram for was found to be satisfactory on symmetry C-18 (4.6×150mm, 5µ Thermosil column) using mobile phase composed of 60:40%v/v phosphate buffer of pH 3.6 and isopropyl alcohol at a flow rate of 1.0 ml/min. The retention time of Rizatriptan was found to be 7.924 min. The system suitability parameters proved that the proposed method is suitable for estimation of Rizatriptan. Tailing factor for the peak was found to be 1.22 and the theoretical plates for separation were found to be 3563. The method was found to be linear in the range of 10-50µg/ml. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD and LOQ were found to be 0.024µg/ml and 0.08 µg/ml respectively. The proposed RP HPLC method was found suitable for the estimation of Rizatriptan in formulations and is simple, selective, reproducible and accurate with good precision and can be successfully applied to routine analytical purpose.

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Introduction

Rizatriptan benzoate is N,N-dimethyl-2-[5-(1H -1,2,4-triazol-1- ylmethyl)-1H-indol-3-yl] ethanamine. It is an anti migraine drug, which selectively activates 5-HT1B/1D receptors. Physical properties are white to off white crystalline powder, soluble in water, melting point 178–180, and stable under ordinary condition.[1] Every year many new drugs and newer drug combinations enter the pharmaceutical area. Analytical methods for these new and first timer drugs are mostly confined only to the manufacturing company. However, availability of multiple analytical methods for the same drug/drug combinations in their formulations is always advantageous. Moreover, development of such methods helps in training the analysts for skilful handling of the sophisticated analytical instruments and the way for research approach. Reference literature and general survey reveals that similar work of development of bioanalytical methods for new drugs and their combinations introduced in the market is continuously underway in many academic institutions.

An exhaustive literature survey on analytical methods led to observations that a very few analytical methods were published for Rizatriptan benzoate and most of the reported methods made use of buffer as the mobile phase in large volume.[2-9] The above observations from literature suggest the requirement for development and validation of new method for estimation of Rizatriptan benzoate with high sensitivity, accuracy, precision, rapid and economic viability. It was therefore hypothesized to develop and validate a suitable method for the estimation of Rizatriptan in dosage form.

Material and Methods

Rizatriptan benzoate pure drug was obtained as a gift sample from Dr. Reddy's, Hyderabad. The pure sample was used without further purification. Tablet formulation (Brand Name-Rizora- manufactured by Intas) was procured from local pharmacy and was used for in the present study. All dilutions were performed in standard class-A, volumetric glassware. All other materials used were of pharmacopoeial grade.

Preparation of standard solution

Rizatriptan benzoate (10 mg) was weighed accurately and diluted with the mobile phase to obtain a stock solution of mg/ml which was further diluted to obtain solution of strength 30µg/ml.

Preparation of Sample Solution

20 tablets were weighed and triturated using mortar and pestle. Tablet powder equivalent to 10 mg of Rizatriptan was accurately weighed and transferred into a 100 ml volumetric flask. 50 ml of diluent was added to the flask and sonicated to dissolve the drug completely and volume was made up to the mark with the dilutent.

Method development[10]

Trials using variation in mobile phase pH and ratio were carried out on C-18 column at 217 nm detection wavelength and 1 ml/ min flow rate and the peak area, shape and retention time were observed.

Validation of the method[11,12]

System Suitability

Six replicates of the Rizatriptan standard was injected into HPLC column and eluted the using optimized conditions. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Specificity

Solutions of standard and samples were prepared as per test procedure and injected into the HPLC system. A study to establish the interference of blank was conducted. Only the diluent was injected into HPLC system in order to check for any peak due to the diluent.

Linearity

Various concentration (10-50 ppm) of the stock solution (1mg/ml) of standard were prepared and analysed using the proposed method in order to check for the linear range of analysis.

Accuracy

Accurately weighed quantity of Rizora tablet powder equivalent to 10 mg was carefully transferred into a 25 ml volumetric flask and 20ml of diluent was added. The mixture was sonicated to dissolve the drug completely. The volume was made up to the mark with the diluent. 5 ml of this solution was further diluted to 25 ml in a volumetric flask with the diluent. 1.5 ml of the above solution was carefully pipette out and diluted to10 ml with the diluents. To this solution was added in three different portions 80, 100 & 120% of standard solution and the resulting sample was analyzed for drug concentration (recovery).

Precision

The precision of the method was validated by checking the repeatability and inter-day repeatability (intermediate precision). The % RSD was calculated.

Robustness

Deliberate changes in the flow rate & mobile phase composition were made and the drug was assayed using the proposed conditions, for determining the robustness of the method.

Limit of Detection

1 ml of the standard solution (30µg/ml) was pipetted and diluted up to 10 ml with the diluent. 0.08 mL of this was pipetted into a 10 ml of volumetric flask and diluted up to the mark.

Limit of Quantification

1 ml of the standard solution (30µg/ml) was pipetted and diluted up to 10 ml with the diluent. 0.27 mL of this solution was carefully pipetted into a 10 ml of volumetric flask and diluted up to the mark.

Results and Discussion

Selection of Wavelength for detection

The absorption maximum of Rizatriptan was determined by UV spectroscopy and was selected for detection of the drug eluted from the HPLC column. The maximum absorption in phosphate buffer pH 3.6 was found to be 217 nm (Figure 1).

Optimization of experimental conditions

The optimization of the chromatographic conditions of elution of Rizatriptan was performed by trying out different compositions of the mobile phase and observing the peak for its proper shape and the retention time (Figure 2, Table 1).

Validation of the method

System Suitability

The system suitability from the six replicate injections of Rizatriptan by observing the % RSD for the retention times (should be not more than 2.0 %), the number of theoretical plates (N) (should be not less than 2000), and tailing factor (T) (should be not more than 2.0). The system suitability studies made it evident that all the parameters were within prescribed limits (Table 2). Hence it could be inferred that the instrument, reagents and column were suitable to perform the assay of the drug.

Specificity

The chromatograms obtained by injecting the standard solution, sample solution as well as the blank (mobile phase) did not exhibit any other significant peaks in addition to the peak of Rizatriptan. Hence it was concluded that the developed method is specific in nature.

Linearity and Range

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The linearity of the method was determined by analysis of standard plots associated with five point standard calibration curve. The peak area obtained from each preparation level of the Rizatriptan standard solution was recorded. A correlation coefficient of not less than 0.9990 was considered as significant to ascertain the linearity and range of the method. The standard calibration curve is represented in the Figure 3.

Accuracy

The accuracy of a method is the closeness of results obtained using the analytical method to the exact value. Accuracy was studied using recovery method (by spiking the sample with known concentration of the standard) and the concentration of the same is determined using the method under consideration. The mean % recovery of drug at each spike level should be not less than 98.0 % and not more than 102.0 %. The method passed the test for accuracy, as the percentage recovery was found to be 100.13 % with a RSD of 0.487 % (Table 3).

Precision

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample. The precision was calculated as the percentage coefficient of variation (% CV) or Relative standard deviation (% RSD). The method passed the test for repeatability as the % RSD was less than 2% (0.88524687 %).

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase and temperature. Effects of variation in the flow rate (±1 ml/min) were studied at three different concentrations and temperatures (±1° C to ±5°C) were studied the results are given in tables 4 & 5. Effect of variation in the different mobile phase ratio also studied at three different levels and the results are given in table 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the signal to noise ratio method. The average base line noise obtained from the blank run (mobile phase injection) was found to be 47 µV whereas the signal obtained from LOD solution (0.08% of target assay concentration) was found to be 136 µV. The S/N value was calculated to be 2.89. The S/N Ratio value shall be 3 for LOD solution.

The signal obtained from the LOQ solution (0.27% of target assay concentration) was found to be 448 µV. The S/N value was calculated to be 9.53. The S/N Ratio value shall be 10 for LOQ solution.

Application of the method to marketed formulation

The developed and validated method was applied for the analysis of the two different marketed formulations of Rizatriptan. The percentage recovery of Rizatriptan 5 mg and Rizatriptan 10 mg was found to be 99.86 % and 99.84 % respectively.

Conclusion

The investigation resulted in the development of a new RP – HPLC method for the estimation of Rizatriptan benzoate in bulk and in formulations. The method is simple, selective, reproducible and accurate with good precision and can be used for routine pharmaceutical analysis.

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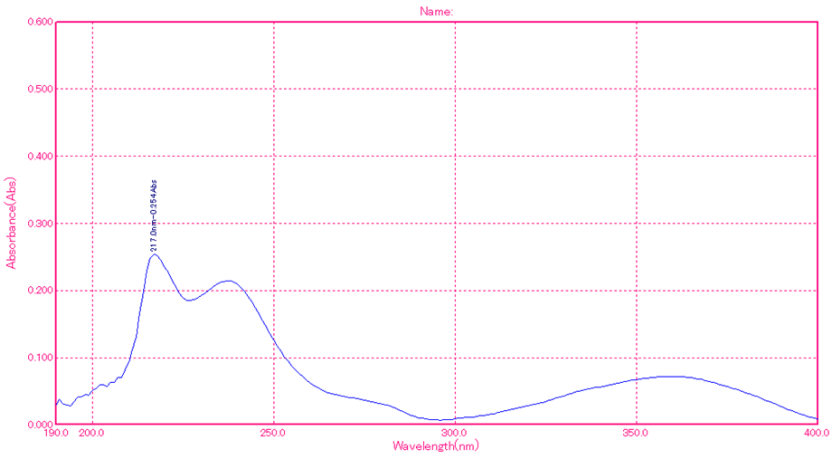

Figure 1 Absorption maxima of Rizatriptan.

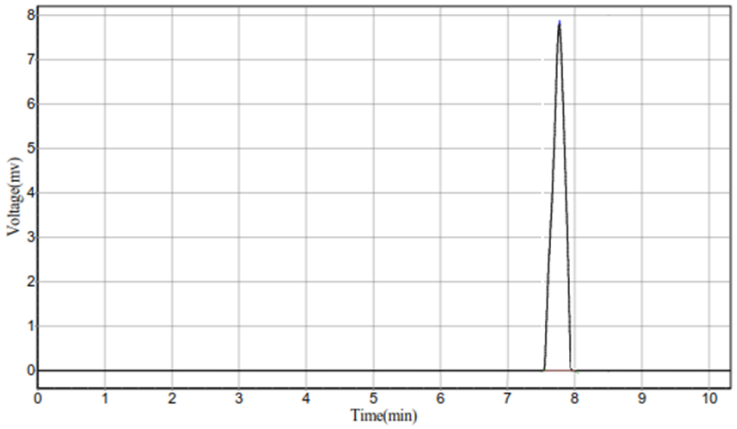

Figure 2 Chromatogram of Rizatriptan in optimized experimental conditions.

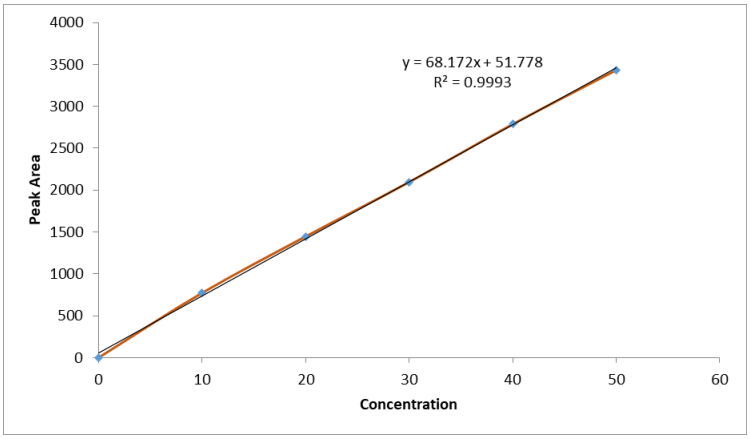

Figure 3 Calibration curve of Rizatriptan.

Table 1 Optimization of experimental conditions.

Mobile Phase	Composition	Retention time	Remark
phosphate buffer (pH 6.3) : Isopropyl alcohol	55:45	-	Peak not found
phosphate buffer (pH 3.6) :	50:50	7.918	Peak shape not
phosphate buffer (pH 3.6) : Isopropyl alcohol	60:40	7.924	Good Peak

Table 2 System suitability parameters

System suitability parameters	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Mean
AUC X 10^6	1.257	1.261	1.267	1.27	1.283	1.267	1.2675
Retention Time	7.924	7.925	7.924	7.923	7.925	7.921	7.924
Tailing Factor	1.22	1.27	1.29	1.14	1.16	1.24	1.220
No. of Theoretical plates	3560	3571	3567	3565	3557	3561	3563.500

Table 3 Accuracy results of the method

Conc. of drug in tablet sample (µg/ml)	Conc. of drug added to final (µg/ml)	% Recovered (mean)
12	24	99.82
12	30	99.87
12	36	100.69
	Mean Recovery	100.13
	SD	0.488
	%RSD	0.487

Table 4 Effect of variation in flowrate (-0.1ml/min).

Concentra-tion (µg/ml)	Retention time (min)*	Peak Area*	Standard deviation	% RSD
20	7.927	1450.87	28.45	1.961
30	7.931	2064.71	20.38	0.987
50	7.93	3317.19	12.17	0.367

Table 5 Effect of variation in flow rate (+0.1 ml/min).

Concentra-tion (µg/ml)	Retention time (min)*	Peak Area*	Standard deviation	% RSD
20	7.914	1439.52	21.361	1.484
30	7.917	2075.33	27.56	1.328
50	7.918	3341.68	36.11	1.081

*****Average of six readings

Table 6 Effect of variation in composition of mobile phase ratio.

Mobile Phase ratios	Retention time (min)*	Peak Area*	Standard deviation	% RSD
25-75	7.917	1457.18	28.63	1.965
30-70	7.917	1449.67	29.733	1.901
35-65	7.92	1451.06	29.654	1.839

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