

“Analytical Method Development and Validation for Quantitative Estimation of Rizatriptan Benzoate”

V Sirisha*, C Sreedhar and T Sreenivasa Rao

Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore-560 064, Karnataka, India

Abstract

A new method was developed for the estimation of Rizatriptan benzoate in bulk and dosage forms by reverse phase HPLC. Gradient reverse phase Chromatography was performed using a C18 column of particle size 5 microns. Mobile phase used was phosphate buffer of 2.5 pH and methanol in the ratio of 70:30. The flow rate was optimized to 0.8 mL/min and the effluent monitored at a wavelength of 227 nm. The retention time was observed at 4.1 min for Rizatriptan benzoate. The standard curve was linear over a working range of 1-10 µg/mL and gave an average correlation factor of 0.9998. The limit of detection and the limit of quantization were found to be 0.96 µg and 3.21 µg respectively. The method showed good recoveries 100.025% (98%-102%) and relative standard deviations of intraday (0.90) and inter day (0.91) precision were less than 1. This method can be conveniently utilised for routine analysis of Rizatriptan benzoate.

Keywords: Rizatriptan benzoate; RP-HPLC

Introduction

Rizatriptan benzoate (Figure 1) is a triptan drug used for the treatment of migraine headaches. It is a selective 5-Hydroxytryptamine 1 receptor subtype agonist [1]. Since there are only few methods are available for the determination of Rizatriptan benzoate. The present work is an attempt to estimate the same by a New HPLC method.

The literature review shows very few methods for the determination of Rizatriptan benzoate and pharmaceutical validations by HPLC method but that various other methods like UV spectroscopic method for Rizatriptan benzoate [2,3], HPLC method for Rizatriptan benzoate [4-6], LC-MS and LC-MS/MS method for determination of Rizatriptan benzoate in human plasma [7-9].

This method can be successfully used for routine analysis of Rizatriptan benzoate as it is rapid, simple, selective and sensitive method for the determination using High Performance Liquid Chromatographic (HPLC) technique.

Materials and Methods

Analytically pure sample of Rizatriptan benzoate procured as gift sample by Dr. Reddy's laboratories (Hyderabad). HPLC grade Methanol (Merck), HPLC grade water and GR grade orthophosphoric acid 88% was used. Pharmaceutical formulation RITZA tablets (label claim 5 mg) batch no. 401874, Mfg. Lic. No. 164/MN/AP/95/F/R Manufactured by NATCO Pharma Limited, was used in the HPLC.

Apparatus and chromatographic conditions

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (4 MPa or 40 barr), rheodyne injector, UV variable detector, and Agilent syringe (50 µl) was used. The separations were achieved on Water's XBridge™ column 5 µm 4.6×250 mm with UV detection at 227

nm. EZChrome Elite software was used. The mobile phase phosphate buffer: methanol with gradient elution at a flow rate of 0.8 ml/min at retention time 4.1 min gave symmetric peak.

Stock solution and standards

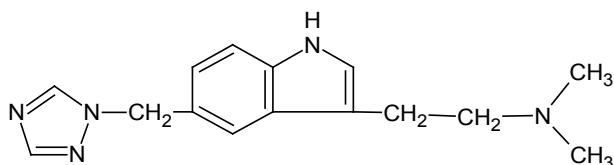
25 g of Rizatriptan benzoate standard was dissolved in 10 ml of methanol and sonicate for 10 mins and make up with methanol to give a solution containing 1000 µg/ml. From this stock solution, pipetted out 5 ml, placed in to 50 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100 µg/ml. From the above 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of solutions were pipetted out into two separate 10 mL volumetric flasks and volume was made up to the mark with the diluents used. This gave the concentration of 1, 2, 4, 6, 8 and 10 µg/ml.

Preparation of sample solution

An accurately weighed quantity of the powder equivalent to 5 mg of Rizatriptan benzoate was benzoate was transferred to 25 ml volumetric flask containing 10 ml of diluents. The contents of the flask were sonicated for 15 min and made up to 25 ml with diluents. The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter. 20 µl of each concentration was injected and the peak was observed at 4.1 minute (Rt). Chromatogram of blank is shown in figure 2.

Validation

Linearity: Linearity test solutions were prepared from the stock solution at six concentration levels 1, 2, 4, 6, 8 and 10 µg/ml. The peak area versus concentration data was performed by least-squares linear regression analysis. The linearity range was found to be 1-10 µg/ml. The standard calibration curve for rizatriptan benzoate was constructed



Rizatriptan

Figure 1: Structure of Rizatriptan benzoate.

*Corresponding author: V. Sirisha, Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore-560 064, Karnataka, India, Tel: 91 8951325170; E-mail: vana.sirisha@gmail.com

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using the peak-area versus the nominal concentrations of the analytes which is shown in figure 3.

The results of linearity are shown in the table 1. Figure 4 shows the linearity chromatogram for rizatriptan benzoate (Figure 5).

Accuracy: The accuracy of the assay method was evaluated in triplicate at three concentration levels, 50,100 and 150%. The percentage recovery values were in between 100-100.5 which indicates the method was accurate. Accuracy/recovery experiments were performed in triplicate (Table 2).

The results of accuracy are shown in the table 2 and chromatograms for accuracy are shown in the figure 3.

Precision: The precision of method was ascertained from the peak area response obtained by actual determination of six replicates of a fixed amount of drug. The percent relative standard deviations were calculated and it was found that % RSD is less than 2% which indicates the proposed method has good reproducibility. The results of precision are shown in the table 3.

LOD and LOQ: The LOD and LOQ for Rizatriptan were estimated by using the formula

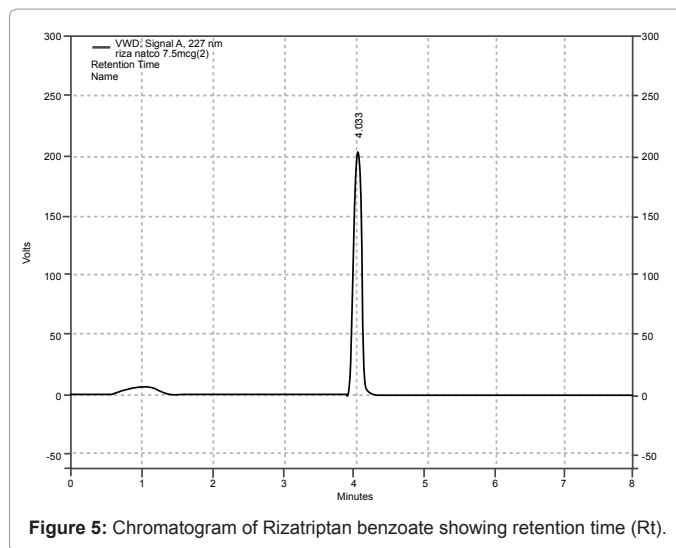


Figure 5: Chromatogram of Rizatriptan benzoate showing retention time (Rt).

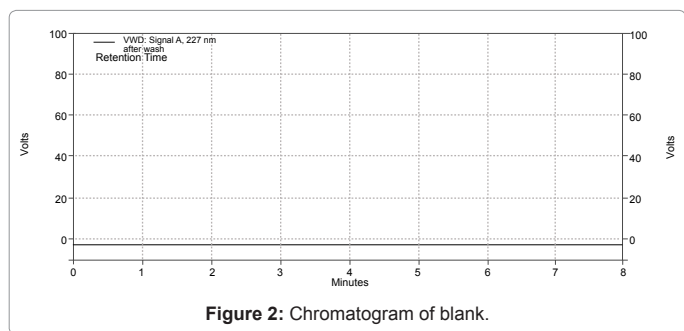


Figure 2: Chromatogram of blank.

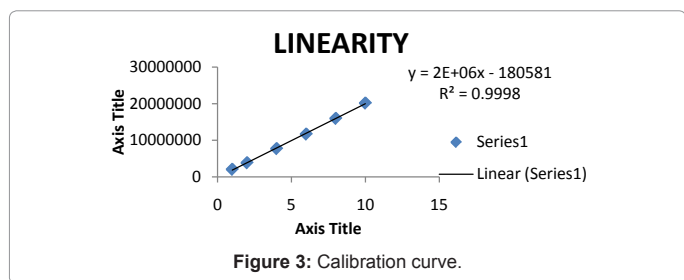


Figure 3: Calibration curve.

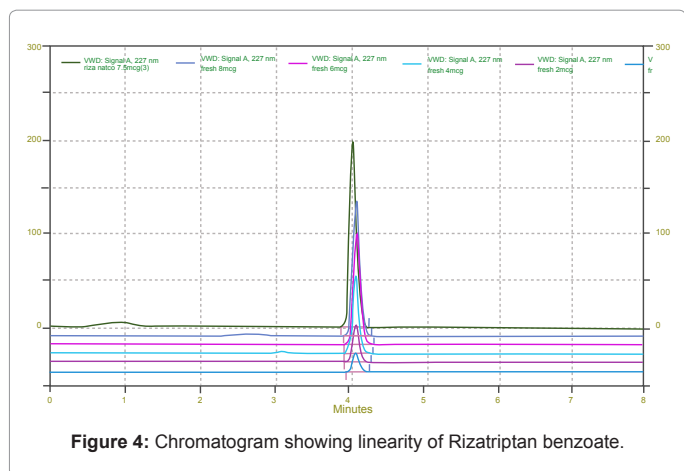


Figure 4: Chromatogram showing linearity of Rizatriptan benzoate.

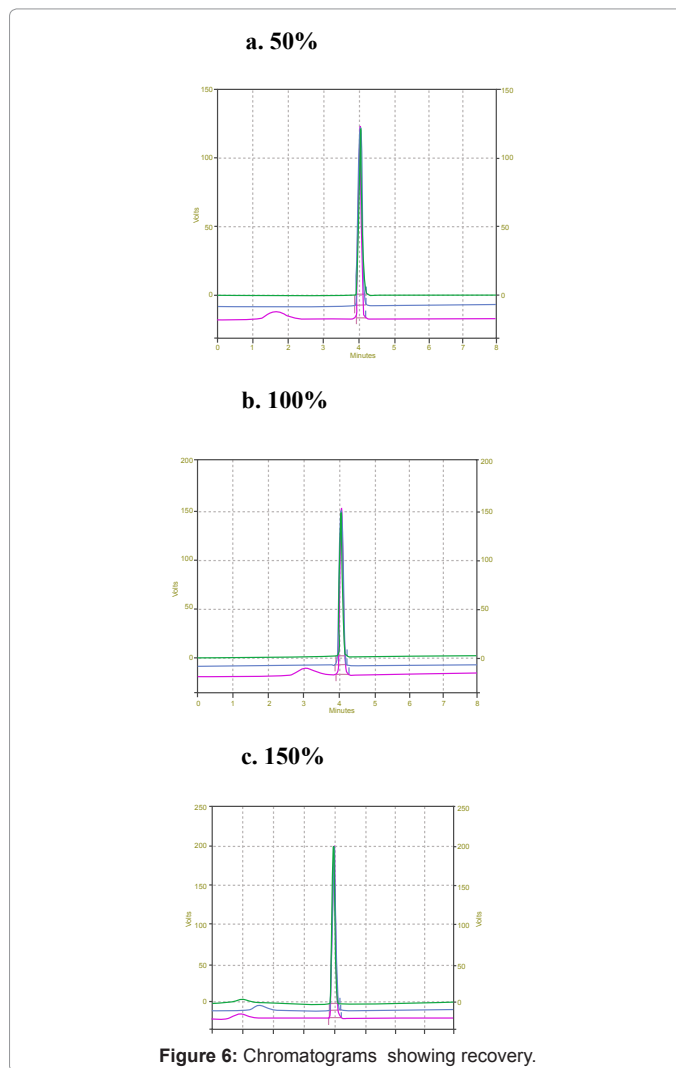


Figure 6: Chromatograms showing recovery.

$$3 \times \frac{\text{standard deviation}}{\text{slope}} \text{ and } 10.1 \times \frac{\text{standard deviation}}{\text{slope}}$$

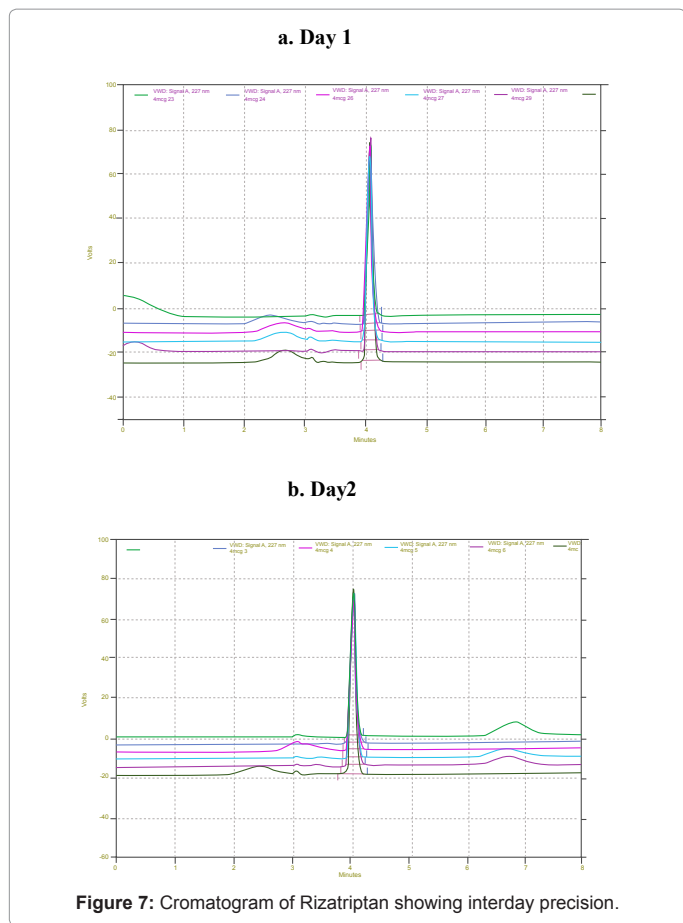


Figure 7: Chromatogram of Rizatriptan showing interday precision.

respectively, by injecting a series of dilute solutions with known concentration.

LOD was found to be 0.9 ppm.

LOQ was found to be 3.2 ppm.

Results

The objective of the proposed work was to develop a method for the determination of Rizatriptan benzoate and to validate the methods according to ICH and USP guidelines and applying the same for its estimation in pharmaceutical formulations (Figure 9).

Drug was eluted by using different mobile phases like glacial acetic acid and ortho phosphoric acid, different pH like 2-8 and organic modifiers like methanol and acetonitrile.

The chromatographic separation was achieved on a stainless steel column, symmetry (4.6×150 mm) column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron. The developed method was validated. Table 4 shows the optimized parameters for Rizatriptan benzoate and system suitability parameters are listed in table 5.

Conclusion

- The proposed method was found to be simple, precise, accurate and rapid for determination of Rizatriptan benzoate, in pure form.
- The mobile phase is simple to prepare and economical.
- The sample recoveries in all formulations were in good agreement within the limit.

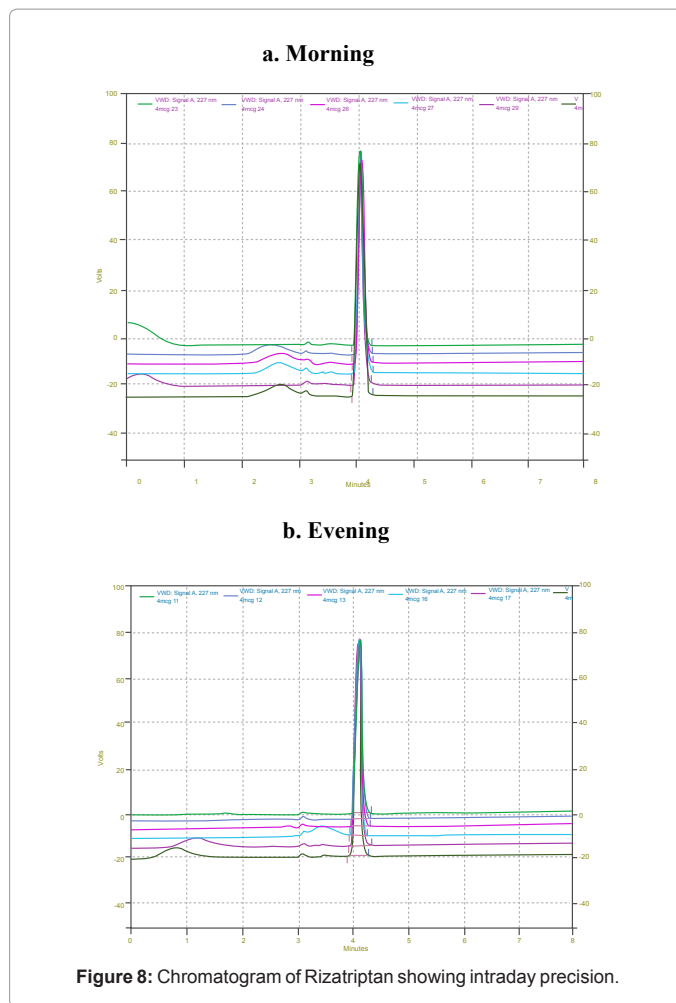


Figure 8: Chromatogram of Rizatriptan showing intraday precision.

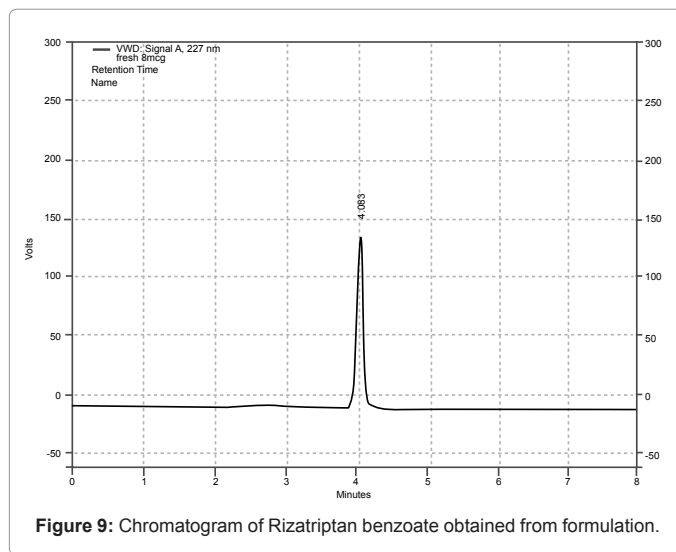


Figure 9: Chromatogram of Rizatriptan benzoate obtained from formulation.

- Hence, this method can be easily and conveniently adopted for routine analysis of Rizatriptan benzoate.

Acknowledgement

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