CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1490068

Available online at: http://www.iajps.com Research Article

DEVELOPMENT OF A NEW CHROMATOGRAPHIC METHOD FOR ESTIMATION OF RIZATRIPTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Kambampati Pavani Sai Durga Mounika[1*], Dr.M.Dhanalakshmi[2], P. Deepthi[3]
Department of Pharmaceutical Analysis, KLR Pharmacy College, Bhadradri Kothagudem, Telangana, India.

Abstract:
A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Rizatriptan, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Xterra RP 18 (4.6 x 250mm, 5µm) column using a mixture of Methanol: Water (75:25% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 262 nm. The retention time of the Rizatriptan was 5.481 min. The method produce linear responses in the concentration range of 15-75ppm of Rizatriptan. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Rizatriptan, RP-HPLC, Validation, Linearty, ICH guidelines*

***Corresponding Author:**
K.Pavani Sai Durga Mounika,
**Department of Pharmaceutical Analysis,*
KLR Pharmacy College,
Bhadradri Kothagudem,
Telangana, India.
E-mail: psd.mounika@gmail.com

QR code

Please cite this article in press K.Pavani Sai Durga Mounika et al., ***Development of a New Chromatographic Method for Estimation of Rizatriptan in Bulk and Pharmaceutical Dosage Form.,*** *Indo Am. J. P. Sci, 2018; 05(11).*

INTRODUCTION:

Rizatriptan is an antimigraine drug used to treat migraines[1]. Three distinct pharmacological actions have been implicated in the antimigraine effect of the triptans: (1) stimulation of presynaptic 5-HT1D receptors, which serves to inhibit both dural vasodilation and inflammation; (2) direct inhibition of trigeminal nuclei cell excitability via 5-HT1B/1D receptor agonism in the brainstem and (3) vasoconstriction of meningeal, dural, cerebral or pial vessels as a result of vascular 5-HT1B receptor agonism. The literature survey reveals that HPLC[2], Liquid chromatography – Electrospray Tandem Mass spectrometry (LC-MS)[3,4], Liquid chromatographic method[5], Spectrofluorimetric[6], Spectrophotometric[7-12], RP-LCDAD[13], Electrokinetic Capillary Chromatography[14] and only few methods were reported for RP-HPLC[15,16] for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC method for the determination of Rizatriptan in API and formulation. The developed method validated as per ICH guidelines17[-19]. It is chemically known as N, N-dimethyl-2-[5-(1H-1, 2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine.

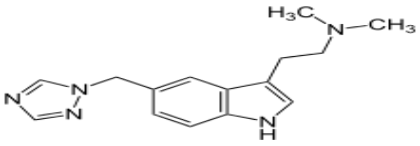

Figure 1: Structure of Rizatriptan

MATERIALS AND METHODS:

Chemicals and reagents used

Rizatriptan as pure standard reference drug was obtained from Sura labs, Hyderabad, India. Acetonitrile, Water and Methanol used were of HPLC grade and purchased from Merck specialties Private Limited, Mumbai, India.

Chromatographic conditions and instrumentation

HPLC analysis was performed on WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. Chromatographic conditions were cited in Table 1.

Table.1: Chromatographic conditions for Rizatriptan

Mobile phase ratio	Methanol:water (75:25% v/v)
Column	X-Terra RP18 (4.6×250mm) 5µ
Column temperature	30ºC
Wavelength	262nm
Flow rate	1ml/min
Injection volume	10µl
Run time	10minutes

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Rizatriptan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.45ml of the above Rizatriptan stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 75:25 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. X Terra RP 18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of mobile phase:

Accurately measured 750ml (75%) of Methanol and 250ml of Water (25%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method development

Trials showed that mobile phase with X-Terra RP18 (4.6×250mm) 5µ column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Rizatriptan as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Rizatriptan was recorded in Figure 2. Now the sample solution was injected separately and chromatogram was recorded in figure 3 until the reproducibility of the peak areas were satisfactory.

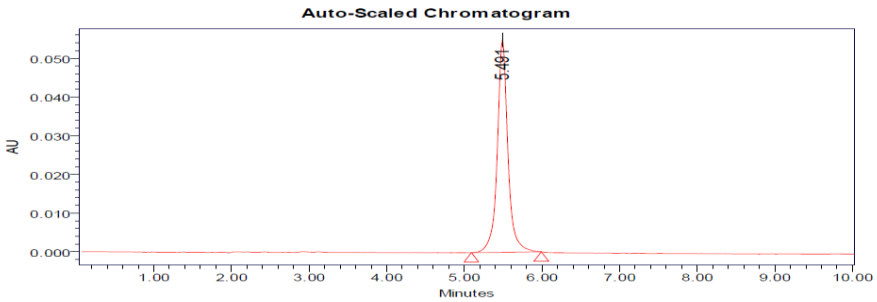

Figure 2: Standard Chromatogram of Rizatriptan

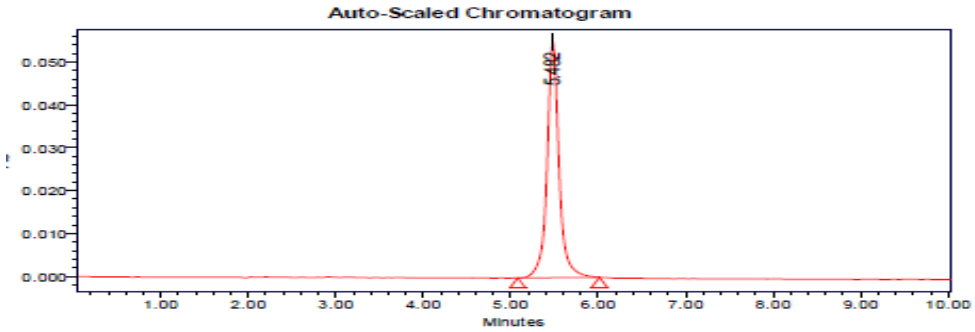

Figure 3: Sample Chromatogram of Rizatriptan

Analytical method validation

HPLC method was validated according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity

Inject each level (15, 30, 45, 60 and 75µg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area in the concentration range of 15 – 75µg/mL. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different analysts by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Rizatriptan and calculate the individual recovery and mean recovery values.

Robustness

Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

System suitability

This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Rizatriptan and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

It is calculated by the formula

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\mathbf{LOQ = 10 \times \sigma / S}$$

RESULTS AND DISCUSSION:

Linearity and range

Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of 15, 30, 45, 60 and 75µg/mL solutions (prepared from standard stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.99, and the intercept 12414. These values meet the validation criteria as shown in Figure 4 and linearity values tabulated in Table 2.

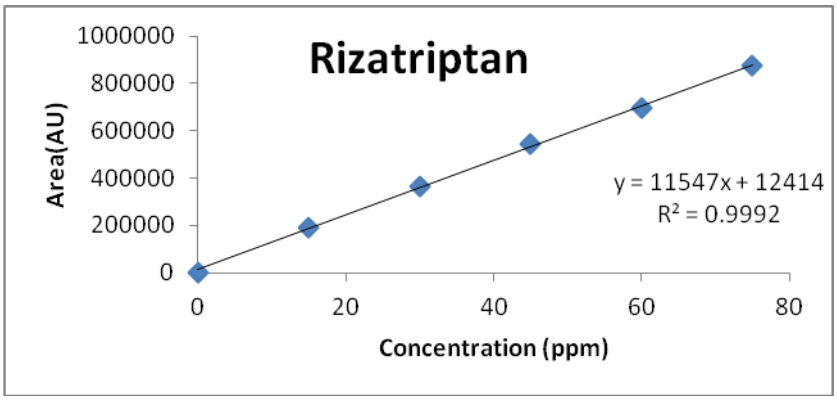

Figure 4: Calibration curve of Rizatriptan

Table 2: Chromatographic data for linearity study

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	15	192423
66	30	366108
100	45	541715
133	60	698851
166	75	873452

Precision

Intermediate precision

Analyst 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results were reported in Table 3.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results were reported in Table 4.

Table-3: Results of Intermediate precision Analyst 1 for Rizatriptan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rizatriptan	5.352	516091	54804	9009.0	1.1
2	Rizatriptan	5.346	518221	54903	9131.5	1.1
3	Rizatriptan	5.306	519536	55996	9071.7	1.0
4	Rizatriptan	5.284	519881	56102	9015.7	1.0
5	Rizatriptan	5.319	519895	55577	8987.3	1.0
6	Rizatriptan	5.306	522826	55808	9070.5	1.0
Mean			519408.3			
Std. Dev.			2216.8			
% RSD			0.4			

Table-4: Results of Intermediate precision Analyst 2 for Rizatriptan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rizatriptan	5.274	518217	55506	8953.2	1.1
2	Rizatriptan	5.306	518821	54903	9131.5	1.1
3	Rizatriptan	5.306	518821	54903	9131.5	1.1
4	Rizatriptan	5.274	518217	55506	8953.2	1.1
5	Rizatriptan	5.352	516091	54804	9009.0	1.1
6	Rizatriptan	5.319	519895	55577	8987.3	1.0
Mean			518343.7			
Std. Dev.			1262.452			
% RSD			0.24			

Repeatability

Preparation of Rizatriptan Product Solution for Precision:

Accurately weigh and transfer 10 mg of Rizatriptan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.45ml of the above Rizatriptan stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were reported in Table 5.

Table -5: Results of repeatability for Rizatriptan:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rizatriptan	5.352	516091	54804	9009.0	1.1
2	Rizatriptan	5.346	518821	54903	9131.5	1.1
3	Rizatriptan	5.293	519536	55996	9071.7	1.0
4	Rizatriptan	5.284	519881	56012	9075.7	1.0
5	Rizatriptan	5.319	519895	55577	8987.3	1.0
Mean			518844.8			
Std.dev			1599.873			
%RSD			0.3			

Accuracy:
Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated. The results were reported in Table 6.

Table -6: The accuracy results for Rizatriptan

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	269654.7	22.5	22.43	99.6%	99.0%
100%	529274	45	44.2	98.2%	
150%	794469.3	67.5	67.1	99.4%	

Robustness
The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rizatriptan. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard sample of Rizatriptan was injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were reported in Table 7.

Table 7: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	530529	5.491	9222	1.03
Less Flow rate of 0.9 mL/min	566441	5.599	9364	1.02
More Flow rate of 1.1 mL/min	459187	4.576	7559	0.98
Less organic phase	24366	7.415	12009	1.00
More organic phase	93382	4.576	8274	1.07

System suitability
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were cited in table 8.

Table -8: Results of system suitability for Rizatriptan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rizatriptan	5.395	514884	54648	9011	1.07
2	Rizatriptan	5.484	530529	55564	9222	1.05
3	Rizatriptan	5.491	521608	54920	9148	1.04
4	Rizatriptan	5.482	522448	54873	9186	1.06
5	Rizatriptan	5.491	521608	54920	9148	1.04
Mean			522215.4			
Std. Dev.			5560.066			
% RSD			1.06			

Specificity
The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The % purity of Rizatriptan in pharmaceutical dosage form was found to be 100.4%. Analytical method was tested for specificity to measure accurately quantitates Rizatriptan in drug product. The results were reported in Table 9 and 10.

Table-9: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Rizatriptan	5.427	530023	56127	1.03	9118	1
2	Rizatriptan	5.430	531649	56299	1.05	9364	2
3	Rizatriptan	5.443	533969	55991	1.05	9186	3

Table 10: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Rizatriptan	5.453	534995	55722	1.05	9124	1
2	Rizatriptan	5.462	532954	56050	1.03	9207	2
3	Rizatriptan	5.466	533577	56095	1.03	9235	3

Limit of detection

Limit of detection is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. It is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 1.5µg/ml of Rizatriptan.

Limit of quantification

Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was found to be 4.7µg/ml of Rizatriptan.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Rizatriptan in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Rizatriptan was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP- HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Rizatriptan in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT:

Authors express their sincere thanks to our principal Dr. M. Dhanalakshmi, M.Pharm,Ph.D. KLR Pharmacy College, Palvoncha and the Management of KLR Pharmacy College for providing necessary facilities to carry out the research work.

REFERENCES:

1. Oldman AD, Smith LA, McQuay HS, Moore RA. Pharmacological Treatments for Acute Migraine: Quantitative Systematic Review. Pain 2002; 97(3): 247–257.
2. Jocic B, Zecevic M, Zivanovic L and Licanski A. A chemometrical approach to optimization and validation of an HPLC assay for Rizatriptan and its impurities in tablets. Analytical Letters. 2007;40(12): 2301-2316.
3. Vishwanathan K, Bartlett MG, Stewart JT. Determination of Antimigraine Compounds Rizatriptan, Zolmitriptan, Nortriptan and Sumatriptan in Human Serum by Liquid Chromatography/ Electrospray Tandem Mass Spectrometry. Rapid Commun. Mass Spectrom. 2000; 14(13): 168–172.
4. Guo J, Zhang A, Zhao L, Sun X, Zhao Y, Gao H, Liu Z and Qiao S. Determination of rizatriptan in human plasma by Liquid Chromatographic

Eletrospray Tandem Mass Spectrometry: Application to a pharmacokinetic study. Biomedical Chromatography. 2006;20(1):61-66.
5. Chen J, Jiang X, Jiang W, Mei N, Gao X and Zhang Q. Liquid chromatographic method for the determination of Rizatriptan in human plasma. Journal Chromatography B. 2004;805(1):169-173.
6. Altinoz S, Ucar G and Yıldız E. Determination of rizatriptan in its tablet dosage forms by UV Spectrophotometric and Spectrofluorimetric methods. Analytical Letters. 2002;35(15):2471-2485.
7. Amol K, Vivek R, Alpana K, Hassan DM, Maria S and Swaroop L. Spectrophotometric method for analysis of Rizatriptan benzoate. International Journal of Pharmaceutical Sciences. 2009;1(2):307-309.
8. Kumari AS, Subhasish S, Kaushik DK and Annapurna MM. UVspectroscopic methods for estimation of Rizatriptan benzoate in pharmaceutical preparations. International Journal of ChemTech Research. 2010;2(1): 653-659.
9. Vivek R, Amol K, Alpana K, Hassan DM, Zaria S and Swaroop L. Spectrophotometric estimation of Rizatriptan benzoate. Asian Journal of Research in Chemistry. 2010;3(1):175-177.
10. Devprakash, Senthilkumar GP, Prithviraj SY and Mani TT. Determination of Rizatriptan in bulk and its tablet dosage forms by UV Spectroscopic method. International Journal of Pharmaceutical Sciences and Research. 2011;2(8):2041-2044.
11. Shanmukha kumar JV, Prasad KRS, Ramachandran D and Settaluri VS. Development and validation of Spectrophotometric methods for determination of maxalt (Rizatriptan benzoate) in pure and pharmaceutical formulation. Journal of Analytical Chemistry. 2011;1 (5):1-5.
12. Gowry SD and Vamsi KM. Visible spectrophotometric methods for the determination of Rizatriptan in pure form and in pharmaceutical formulations. Analytical Chemistry. An Indian journal. 2007; 3(4-6):4-6.
13. Zecevic M, Jocic B, Zivanovic L and Protic A. Application of multicriteria methodology in the development of improved RP-LCDAD for determination of Rizatriptan and its degradation products. Chromatographia. 2008;68(11-12): 911-918.
14. Mahuzier PE, Clark BJ, Crumpton AJ and Kevin DA. Quantitative microemulsion electrokinetic capillary chromatography analysis of formulated drug products. Journal of Separation Sciences. 2001; 24(9):784-788.
15. Qin YP, Zou YG, Liang MZ and Yu Q. Determination of rizatriptan in human plasma by RP-HPLC with fluorescence detection. Yaowu Fenxi Zazhi. 2006;26(1):7-9.
16. Sagar PV, Kumar D, Suddhasattya D and Samal HB. Simultaneous estimation of rizatriptan, sumatriptan and zolmitriptan by RP-HPLC method in bulk. Journal of Pharmacy Research. 2010;3(12):2930-2933.
17. ICH Q2A, "Validation of Analytical Methods, Definitions and Terminology", ICH Harmonized Tripartite Guideline, (1999).
18. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and Terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
19. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.