



Development and validation of RP-HPLC method for quantitative analysis of Rizatriptan benzoate in pure and Pharmaceutical formulations

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ABSTRACT

A reverse phase high performance liquid chromatographic method was performed by using Intersil DBC C₁₈ column (250mmX4.6mmX5μ particle size) with UV detection at 225 nm. An isocratic mobile phase consisting of Phosphate buffer pH6.5:Aceto nitrile:Metahnol-87:7.8:5.2 (v/v/v) at a flow rate of 1ml/min.The retention time for Rizatriptan benzoate was 8.435 min. The method was linear in the concentration range of 20-60 μg/ml of Rizatriptan benzoate with the correlation coefficient of 0.999. The method was validated for linearity,accuracy,precision,limit of detection,limit of quantification,robustness and ruggedness.Recovery of Rizatriptan benzoate was found to be 101% to 102%.The developed reverse phase high performance liquid chromatographic method was simple, sensitive, precise and accurate and the method was found suitable for estimating in tablet dosage form.

Keywords: Rizatriptan benzoate, C₁₈ column, Reverse phase, Validation

1. INTRODUCTION

Rizatriptan benzoate is a triptan drug used for the treatment of migraine headaches. It is a selective 5-Hydroxytryptamine 1 receptor subtype agonist¹. 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 RP-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 plasma⁷⁻⁹.

In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1. Standards and chemicals used

Rizatriptan benzoate was provided by Lupin laboratories,Mumbai. All the chemicals Acetonitrile, Methanol, water were HPLC grade, Merck Specialties Private Limited, Mumbai, India. Commercial tablets of Rizatriptan benzoate were purchased from local market .

2.2 Preparation of the mobile phase

Into a 1000ml cleaned volumetric flask, HPLC grade 52ml Methanol,

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acetonitrile 78ml and 870ml of Phosphate buffer pH6.5 (which are filtered through 0.25mm membrane filters by vacuum filtration) were slowly added , mixed well and sonicated upto 20min. Cool the above solution. This solution is again sonicated to 10min. Cool the solution to room temperature and use for chromatography method.

2.3. Preparation of Standard drug solutions

100mg of Rizatriptan benzoate was accurately weighed and is dissolved in few ml of the mobile phase and sonicated for few min to dissolve the drug completely. Then it is filtered through 0.2μ pore filter paper and the volume is made up to 100ml with mobile phase to get a concentration of 1mg/ml stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

2.4. Sample Preparation

20 commercial tablets of Rizatriptan benzoate (Rizact-5mg) were finely powdered and the powder equivalent to 10mg of Rizatriptan benzoate accurately weighed to 50ml volumetric flask and dissolved in few ml of mobile phase. The above solution was subjected to sonication for 15min. After getting clear solution it is filtered through 0.25μm membrane filters and the solution is made up to 50ml with mobile phase resulting in preparation of 10 mg/ml solution. This is further diluted so as to obtain required concentration of Rizatriptan benzoate pharmaceutical dosage form.

2.5. RP-HPLC Method development

Based on nature and solubility characteristics of Rizatriptan benzoate, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C₁₈ column was found to be optimum.

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture Phosphate buffer pH6.5:Aceto nitrile:Metahnlol-87:7.8:5.2(V/V/V) of was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.The chromatographic conditions for the estimation of Rizatriptan benzoate were discussed in table1 .

Table 1. Optimized chromatographic conditions for estimation of Rizatriptan benzoate

Parameter	Condition
Mobile phase	Phosphate buffer pH6.5: Aceto nitrile:Metahnlol-87:7.8:5.2(V/V/V)
Pump mode	Isocratic
pH	5.8
Diluents	Mobile phase
Column	Intersil ODS3v C18 column (250 X 4.6 mm, 5μ)
Column Temp	Ambient
Wavelength	225nm
Injection Volume	20 μl
Flow rate	1.0ml/min
Run time	20minutes

3.0 RESULTS AND DISCUSSION

3.1 Analysis of formulation

The sample solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. The amount of drug present in the pharmaceutical formulation was calculated using standard calibration curve (concentration in μg/ml was taken on X –axis and average peak area on Y –axis). A representative chromatogram has been given in Fig. 1

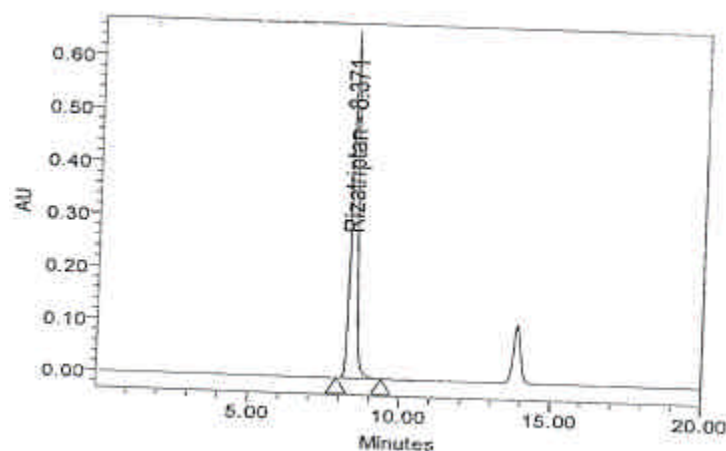


Fig. 1 chromatogram of Rizatriptan benzoate

3.2 VALIDATION OF THE PROPOSED METHOD

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

3.2.1. Linearity

It is the ability of the method to elicit test results directly proportional to analyte concentration with in a given range. Linearity was performed by preparing standard solutions of Rizatriptan benzoate at different concentration levels, twenty micro liters of each concentration was injected in into the HPLC system. The peak responses were read at 225nm and the corresponding chromatograms were recorded. Linearity plots of concentration over peak areas was constructed. Linearity results were obtained in the concentration range of 40-100μg/ml .The results were presented in Table 2.

Table- 2. Linearity results of Rizatriptan benzoate

Concentration of Rizatriptan benzoate in μg/ml	Peak area
20	7059978
30	7714809
40	8569872
50	9224177
60	9728305

3.2.2. Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

Intraday precision

To study the intraday precision, six replicate standard solutions (60μg/ml) of Rizatriptan benzoate were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.96 which are well within the acceptable criteria of not more than 2.0.

Interday precision

To study the interday precision, six replicate standard solutions (60ppm) of Rizatriptan benzoate were injected on three consecutive days .The percent relative standard deviation (% RSD) was calculated and it was found to be 0.76 which are well within the acceptable criteria of not more than 2.0.

3.2.3. Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC(LC2010 A HT), Agilent HPLC. By different operators using different columns of similar type like Hypersil C₁₈ Hichron C₁₈. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, is ruggedness.

3.2.4. Limit of Detection and Limit of Quantification

A Calibration curve was prepared using concentrations in the range of 40-100 μg/ml (expected detection limit range). The standard devia-

tion of Y-intercepts of regression line was determined and kept in following equation for the determination of detection limit and quantitation limit. The results were reported in table 3.

$$\text{Limit of detection} = \frac{\sigma \times 3.3}{S}$$

$$\text{Limit of quantification} = \frac{\sigma \times 10}{S}$$

Where,

σ = the standard deviation of the response.

S = the slope of the calibration curve

Table 3. Limit of Detection and Limit of Quantification for Rizatriptan benzoate

Parameter	Values
Limit of Quantification	3.0 μ g/ml
Limit of Detection	1.0 μ g/ml

3.2.5. Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 50%, 100% and 150% level of 40ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery was calculated and results are presented in table 4. Satisfactory recoveries ranging from 99% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate, there is no interference of additives.

Table 4. Accuracy Recovery Results

Level	Target In μ g/ml	Amount of Rizatriptan benzoate spiked (μ g/ml)	Total in μ g/ml	Amount of Rizatriptan benzoate recovered (μ g/ml)	% Recovery
50%	40	20	60	60.2	100.33
	40	20	60	60.1	100.16
	40	20	60	60.5	100.83
100%	40	40	80	80.1	100.12
	40	40	80	80.3	100.37
	40	40	80	80.4	100.5
150%	40	60	100	100.5	100.51
	40	60	100	100.61	100.61
	40	60	100	100.2	100.2

3.2.6. Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was

robust in nature. The robustness results were mentioned in table 5.1 & 5.2

Table 5.1-Data of effect of variation in flow rate

S.No	Flow Rate (ml/min)	System Suitability results	
		Retention time	USP Tailing
1	0.9(less)	8.250	1.67519
2	1.1(more)	8.345	1.780

Table 5.2 Data of effect of variation in Mobile phase

S.No	Mobile phase Ratio	System Suitability results	
		Retention time	USP Tailing
1	Buffer pH6.5: Aceto nitrile:Metahnol (85:8.8:6.2) (less)	8.396	1.675
2	Buffer pH6.5: Aceto nitrile:Metahnol (89:6.8:4.2) (more)	8.427	1.705

3.2.7. System suitability parameters:

The system suitability parameters like Tailing factor, Theoretical plates are discussed in the table 6.

Table 6. System suitability parameters

Drug	Retention time	Tail factor	Theoretical plates
Rizatriptan benzoate	8.435	1.52	8352

4. CONCLUSION

A convenient, rapid, accurate, precise RP-HPLC method has been developed for estimation of Rizatriptan benzoate. The proposed method followed the ICH guidelines. The proposed method can be used for the routine analysis of Rizatriptan benzoate in bulk preparations of the drug and in pharmaceutical dosage forms without interference of excipients.

5. REFERENCES

1. Goodman & Gilman's The pharmacological basics of therapeutics. 10th ed. McGraw-Hill, medical publishing house 279-281.
2. Kumari AS, Sahoo S, Kaushik DK, Annapurna MM (2010) UV-spectroscopic methods for estimation of Rizatriptan benzoate in pharmaceutical preparations. Int J ChemTech Res 2: 653.
3. Khedkar A, Rajendra V, Kulkarni A, Dehghan MH, Saifee M, et al. (2009) Spectrophotometric Method for Analysis of Rizatriptan Benzoate. Int J Ph Sci 1: 307- 309.
4. Kannapan N, Madukar A, Ganesh A, Naveen Kumar CH, Mannavalan R (2009) Analytical method development and validation of Rizatriptan benzoate tablets by RP-LC. Int J PharmTech Res 1: 1704-1708.
5. Dahiya D, Sumalatha BV, Gaurav S, Yadav PS, Senthil Kumar

- GP (2012) Estimation of Rizatriptan benzoate by RP-HPLC method in bulk and dosage form. *J Pharm* 5: 120-123.
6. Venkateswarlu P, Gajam S (2011) A validated and simplified RP-HPLC of Rizatriptan benzoate from bulk drugs. *J Pharm Biomed Sci*.
 7. Ramakotaiah M, Kanala K, Challa RC, Chandu BR, Bannoth CK (2011) Determination of Rizatriptan in Human Plasma by Liquid Chromatography Stable Isotope Dilution Electrospray MS–MS for Application in Bioequivalence Study. *Chromatographia* 74: 585-592.
 8. Vishwanathan K, Bartlett MG, Stewart JT (2000) Determination of antimigraine compounds Rizatriptan, Zolmitriptan, Naratriptan and Sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14: 168-172.
 9. Guo JF, Zhang AJ, Zhao L, Sun XH, Zhao YM, et al. (2006) Determination of rizatriptan in human plasma by liquid chromatographic-electrospray tandem mass spectrometry: application to a pharmacokinetic study. *Biomed Chromatogr* 20: 61-66.

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