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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RIZATRIPTAN BENZOATE TABLETS BY RP-LC

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ABSTRACT: This paper describes the analytical method suitable for validation of Rizatriptan benzoate by reversed Phase liquid chromatography (RP-LC) method. The method utilized RP-LC (Water 2695 with UV detector) model and a column L1, 250mm × 4.6 mm, 5µ (Inertsil, ODS- 3V, 250mm × 4.6mm, 5µ). The mobile phases were comprised of A, B of Acetonitrile and Buffer pH (6.5).validation experiments were performed to demonstrate System suitability, specificity, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 30-70 µg/ML^{-1}. The method showed good recoveries (96.64 - 97.71%) and the relative standard deviations of intra and inter-day assay were 97.4% and 96.20% respectively. The method can be used for quality control assay of Rizatriptan benzoate.

KEYWORDS: RP-LC, Rizatriptan benzoate, Analytical method, Quality control, validation.

INTRODUCTION

Rizatriptan Benzoate (RZB), (N, N-dimethyl-2-[5-(1, 2, 4-triazole-1-ylmethyl)-1H-indol-3-yl] ethanamine monobenzoate). Rizatriptan is a triptan drug and it is a selective 5-Hydroxy Triptamine1B/1D (5-HT 1B/1D) receptor agonist [1]. Rizatriptan binds with high affinity to human cloned 5-HT1B and 5-HT1D Receptors. Rizatriptan has weak affinity for other 5-HT1 receptor subtypes (5-HT1A, 5-HT1E, and 5-HT1F) and the 5-HT7 receptor, but has no significant activity at 5-HT2, 5-HT3, α- and ß-adrenergic, dopaminergic, histaminergic, muscarinic or benzodiazepine receptors. Current theories on the etiology of headache suggest that symtoms are due to local cranial vasodilatation and/or to the release of vasoactive and pro-inflammatory peptides from sensory nerve ending in an activated trigeminal system [2-6].

After oral doses, peak plasma Rizatriptan concentrations are obtained in about 1 to 1.5 hours depending on the formulation. Bioavailability is about 40% to 45%. Food may daily the to peak plasma concentrations of the tablet formulation by about 1 hr. plasma protein binding is low (14 %). Rizatriptan metabolized primarily by MOA type A to the inactive indole acetic acid derivative. The active metabolite N-monodesmethyl rizatriptan is formed to a minor degree, other mono metabolites are also produced. About 14 % of the indole acetic acid metabolite and 1% as N-monodesmethyl rizatriptan. The plasma half life is about 2-3 hours. Hence a RP – LC method was developed and validated as per ICH guidelines [7]. The literature reveals that various methods for the determination of Rizatriptan benzoate and pharmaceutical validations among these methods are LC-MS and LC-MS/MS [8-13], HPLC method for Rizatriptan benzoate [14-16], Application and Development of Improved RP-LC-DAD for Rizatriptan and its degradation products [17], a method based on LC/MS/MS [18] using the UV detector were reported.

MATERIALS AND METHOD

Apparatus:

The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 2100 (Cyber scan), the HPLC used is of Water 2695 with UV detector. Column used in HPLC is of 250mm × 4.6mm 5µ(Inertsil, ODS-3V, 250 ×4.6 mm, 5µ is suitable) with a flow rate of 1.0 ml/min (Gradient). The mobile phase consists of A & B with mixture of Acetonitrile and the Buffer pH (6.5) at different proportions A & B which are degassed in a

sonicator for about 10 minutes the injection volume is 20µL and the ultra violet detection was at 225 nm.

Reagents and solutions:

Pure sample of Rizatriptan benzoate USP of 220mg and other ingredients such as acetonitrile and water used were of HPLC and milli-q grade. All other chemicals like Ortho Phasphoric acid, Sodium hydroxide, Sodium Di-hydrogen phasphate Di-butyl amine used were of AR grade. Optimized chromatographic conditions are listed in table no.1.

Accurately weigh and transfer 20mg of Rizatriptan benzoate into a 100 mL of volumetric flask, add about 50 mL of diluent, sonicate to dissolve, make up to volume with diluent. Transfer 5.0 mL of the above solution into 20 mL volumetric flask, dilute to the volume with mobile phase and mix well. Filter the solution through the 0.45 µm filter.

Sample preparation:

Weigh and powder the 20 tablets. An accurately weigh and transfer the powder equivalent to 20 mg of Rizatriptan into 100 ml volumetric flask. Add about 50 ml of mobile phase and sonicate for 20 minutes with occasional swirling to dissolve. Cool it and makeup to the volume with mobile phase. Centrifuge the solution at 3000 rpm for 15 minutes.

Transfer the 5 ml of the above supernatant solution into 20 ml volumetric flask, dilute to the volume with mobile phase and mix well. Filter the solution through the 0.45 µm filter.

Linearity & Range:

The Linearity of detector response is established by plotting a graph to concentration versus area of Rizatriptan standard and determining the correlation coefficient. A series of solution of Rizatriptan standard solution in the concentration ranging from about 60% to 140% level of the target concentration (50mcg/ml of Rizatriptan) were prepared and injected into the HPLC system.

Accuracy:

Accuracy for the assay of Rizatriptan benzoate tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Rizatriptan benzoate standard is added at different levels (60%, 80%, 100%, 120% and 140%). The sample were filtered through 0.45µm membrane filter and injected into the chromatographic system.

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as %RSD. The %RSD was found to be 0.60% in the results of precision.

RESULTS AND DISCUSSION

Rizatriptan standard having concentration 50µg/ml was scanned in UV- region between 200-400 nm. λ max of Rizatriptan Benzoate was found to be at 225 nm.

The Rizatriptan Benzoate peak in the sample was identified by comparing with the Rizatriptan standard and the Retention time was found to be around 9.2 minutes.

The estimation of Rizatriptan Benzoate tablets was carried out by RP-HPLC using Mobile phase having a composition of 870 volumes of phosphate buffer, 78 volumes of Acetonitrile and 52 volumes of Methanol. The ratio pH was found to be 6.5. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. The column used was C18 Inertsil ODS 3V (250 mm x 4.6 mm x 5 µ particle size). Flow rate of Mobile phase was 1.0 ml/min, System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates – 6580.8, and tailing factor – 1.21.

The quantitative estimation was carried out on tablet by taking the same concentration as for standard solution and assay result was found to be 97.4%. The calculation spread sheet data regarding quantitative estimation in depicted assay % in the table no.2.

The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.29% which shows that the method is repeatable.

The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method show Method Repeatability 0.60% which shows that the method is precise.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 30 to 70 mcg/ml of target concentration for Rizatriptan standard with a correlation coefficient value is greater than 0.9999. The correlation coefficient of (r^2) = 1, which shows that the method is capable of producing good response in UV-detector.

The Accuracy limit is the % recovery should be in the range of 98.0% to 102.0%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Water 2695 with UV detector)
Column	Inertsil ODS-3V, 250mm×4.6mm, 5µ is suitable
Mobile Phase*	Acetonitrile and Buffer × mixture A & B
Flow rate	1.0 ml/min
Detection	UV at 225 nm
Injection volume	20µl
Temperature column	35°C ± 2°C & 45°C ± 2°C

Table 2: System suitability parameters

Parameter	Rizatriptan Benzoate
Calibration range (µg/ml)	30-70
Theoretical plates	6580.8
Resolution	-
Tailing factor	1.21
Correlation Coefficient(r^2)	1
% Recovery	100.90% - 99.30%
Assay %	97.40%
System Suitability %RSD	0.29%
Method Repeatability %RSD	0.60%

Table 3: Analysis of formulation and recovery studies

Drug	Label claim (mg)	Estimation		Recovery	
		Mg/tablet	% label claim	Amount added µg/ml	% Recovery
Rizatriptan Benzoate	10 mg	499.7	96.64 %	17.7	100.90 (± 2)
Rizatriptan Benzoate	10 mg	499.9	96.69 %	23.31	100.80 (± 2)
Rizatriptan Benzoate	10 mg	499.8	97.62 %	29.48	100.20 (± 2)
Rizatriptan Benzoate	10 mg	499.9	97.56 %	34.97	100.00 (± 2)
Rizatriptan Benzoate	10 mg	500.3	97.95 %	41.15	99.30 (± 2)

CONCLUSION

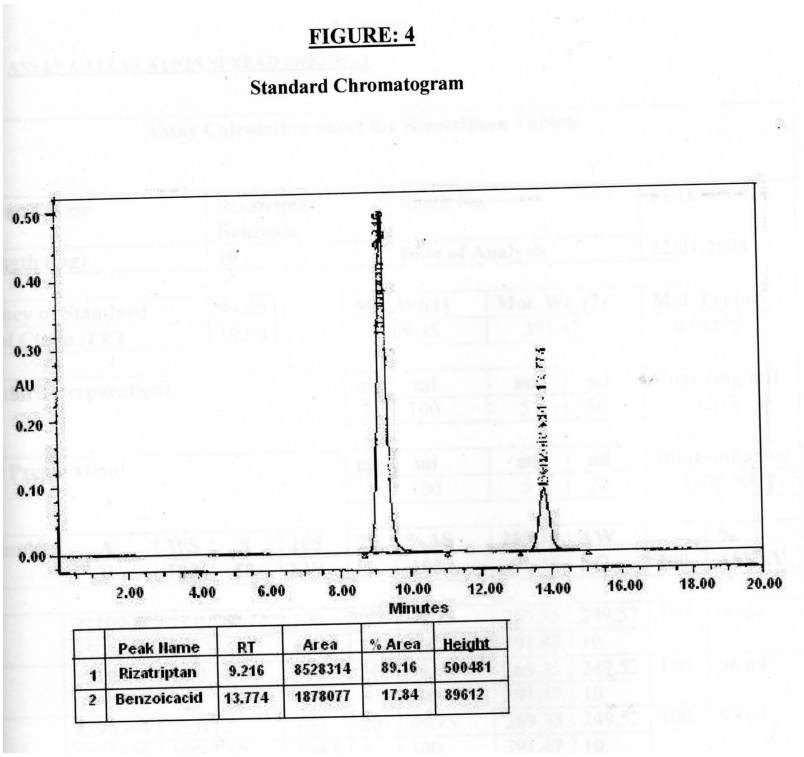
HPLC is at present one of the most sophisticated tools of analysis. The estimation of Rizatriptan Benzoate is done by reverse phase HPLC. The mobile phase consists of buffer (870 volumes of phasphate buffer, 78 volumes of Acetonitrile and 52 volumes of Methanol. The ratio pH was found to be 6.5. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes). The detection is carried out using UV detector set at 225nm. The solutions are chromatographer at the constant flow rate of 1.0 ml/min. The Retention time for Rizatriptan Benzoate was around 9.2 minutes. Linearity range for Rizatriptan Benzoate is 50 to 150 µg/ml.

The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 50µg/ml. the quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the

method. The % recovery 99.30% to 100.90% for Rizatriptan Benzoate.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer's law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision.

CHROMATOGRAM OF STADARD FOR RIZATRITAN BENZOATE



	Peak Name	RT	Area	% Area	Height
1	Rizatriptan	9.216	8528314	89.16	500481
2	Benzoicacid	13.774	1878077	17.84	89612

Figure 1

LINEARITY OF RIZATRIPTAN BENZOATE

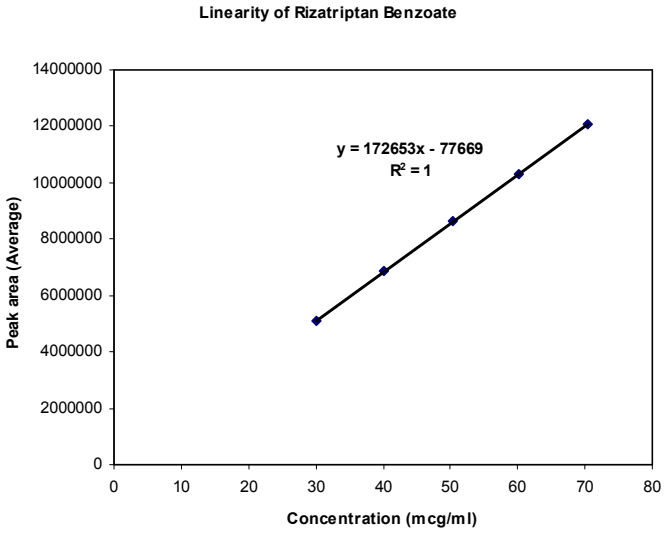


Figure-2

Regression analysis of the calibration curve for Rizatriptan Benzoate showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.9999 in all the curves assayed.

REFERENCES

1. Goldberg MR, Rizatriptan, a novel 5-HT 1B/1D agonist for migraine; Single- and multiple- dose tolerability and pharmacokinetics in healthy subjects, journal of clinical pharmacology. 2000, 40, 74-83.
2. Bou J, Gras J, Cortijo J, Morcillo EJ, Llenas J and Palacios JM Vascular effects of the new anti-migraine agent almotriptan on human cranial and peripheral arteries. 2001, 21**,** 804-812.
3. Bou J, Domenech T, Puig J, Heredia A, Gras J, Fernandez-Forner D, Beleta J and Palacios JM Pharmacological characterization of almotriptan: an indolic 5-HT receptor agonist for the treatment of migraine. *Eur J Pharmacol* , 2000 410**:** 33-41
4. Cabarrocas X and Salvà M Pharmacokinetic and metabolic data on almotriptan, a new antimigraine drug. *Cephalgia,* 1997, 17**,** 421.
5. Dechant KL and Clissold SP Sumatriptan. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the acute treatment of migraine and cluster headache. *Drugs,* 1992, 43**,** 776-798.
6. Arhens S. P. Efficacy and Safety of Rizatriptan water for the acute treatment of migraine, 529.
7. International Conference on Harmonization, Validation of Analytical Procedures; Methodology. Federal Register, Nov. 1996, 1-8.
8. Baumann A, Lohmann W, Schubert B, Oberacher H, Karst U.; Metabolic studies of tetrazepam based on electrochemical simulation in comparison to in vivo and in vitro methods, J Chromatogr A, 2009 Apr 10;1216(15):3192-8. Epub 2009 Feb 10.
9. Stefan Trümpler, Wiebke Lohmann, Bjorn Meermann, Wolfgang Buscher, Michael Sperling and Uwe Karst; Interaction of thimerosal with proteins—ethylmercury adduct formation of human serum albumin and b-lactoglobulin A, Metallomics, 2009, 1, 87-91.
10. Yi Chen, 1, 2 Haijun Miao, 3 Mei Lin, 4 Guorong Fan, 1, 2, Zhanying Hong, 1, 2 Huiling Wu, 1, 2 Yutian Wu1, 2. Development and validation of a selective and robust LC–MS/MS method for high-throughput quantifying rizatriptan in small plasma samples: Application to a clinical pharmacokinetic study**.** *J. Chromatogr.* B 2006**,** 844, 268-277.
11. Ludwig Huber, Validation and qualification in analytical laboratories, First Edition, 1999, 107-140.
12. ICH Harmonized Tripartite Guidelines, Validation of analytical procedure methodology, 6th November, 1996.
13. ICH Harmonized Tripartite Guidelines, Text on Validation of analytical procedure step-4 Q2A, October, 1994.
14. B. Mallikarjuna Rao, Sivaiah Sangaraju, M.K. Srinivasu, P. Madhavan, M. Lalitha Devi, P. Rajendra Kumar; K.B. Chandrasekhar, Ch. Arpitha; T. Satya Balaji. Development and validation of a specific stability indicating HPLC method for rizatriptan benzoate. 1146-1151.
15. HPLC Methods for pharmaceutical Analysis, Vol-2 By, George Lunn, 2000, John wiley & Sons.
16. Lloyd R. Synder, Joseph J. Kirland, Joseph L. Glajch. Practical HPLC method development, 2nd Edition.
17. Mira Zecevic, Biljana Jocic, Ljiljana Zivanovic, Ana Protic. Application of Multicriteria Methodology in the Development of Improved RP-LC-DAD for Determination of Rizatriptan and Its Degradation Products.2006, 911-918.
18. Vyas KP, etal. Disposition and pharmacokinetics of the anti migraine drugs, Rizatriptan, in humans. Drug metabolism and Disposition, 2000, 28, 89-95.

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