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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF RIZATRIPTAN

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ABSTRACT

The main aim of the present work is to develop a selective and precise RP-HPLC method for estimation of Rizatriptan in pure form. Retention time of Rizatriptan was 3.635 min. The developed method was validated according to ICH guidelines. In the range of 2µg to 12µg/mL the linearity of Rizatriptan shows a co-relation coefficient of 0.999. Precision was found to be 1.25. Percentage mean recovery of Rizatriptan was found to be 100.49%. The developed method can be successfully employed for the quality control analysis of Rizatriptan in its pure form.

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INTRODUCTION

Rizatriptan belongs to triptan category and used to treat migraine headaches. Migraine is a common type of headache which interferes with normal functioning. Migraine occurs maybe due to neuronal dysfunction, neuropeptides and inflammatory mediators' local release and Imbalance in the activity of serotonin containing neurons in brainstem nuclei. Rizatriptan is Soluble in water, methanol, 6.8 phosphate buffers, DMSO and DMF. Merck & Co is the Manufacturers of Rizatriptan pure drug in India. Literature survey reveals that few methods were reported for estimation of Rizatriptan (Chandrashekhar *et al.,* 2013; Kishore Kumar *et al.,* 2012; Vishal *et al.,* 2014; Dev Prakash *et al.,* 2012; Sirisha *et al.,* 2013). the present research works aims to develop and validate RP-HPLC method for the quantification of Rizatriptan in bulk.

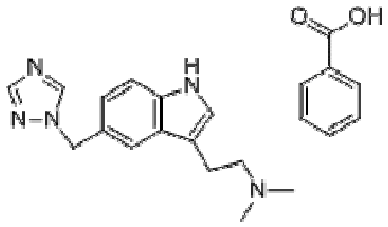

Fig. 1. Rizatriptan chemical structure

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MATERIALS AND METHODS

Instruments

RP-HPLC instrument Shimadzu make, LC-20 AD equipped with a PDA detector was used. Chromatograms were automatically obtained by LC-solution system software.

Reagents and chemicals

Rizatriptan pure drug was procured as gift sample from Varun Herbals, Hyderabad, India. Acetonitrile, Methanol and Water used are of HPLC grade. Potassium phosphate buffer, TEA used is of AR Grade.

Liquid chromatographic conditions

Table.1. The following are the chromatographic conditions used

S.No	PARAMETER	DESCRIPTION
1	Column	Zorbax SB phenyl 250x4.6mm, 5.0µm
2	Flow rate	1.0mL/min
3	Column oven temperature	30^0C
4	Sample temperature	5^0C
5	Detector wavelength	282nm
6	Injection volume	20µl
7	Run time	40min
8	Buffer	1.36g KH_2PO_4 in 1000mL water + 5.0mL TEA, adjusted to P^H 2.5 with OPA.
9	MP-A	Buffer (100%)
10	MP-B	ACN: Methanol: Water(65:15:20)v/v

Gradient program

Table 2. Gradient program for Optimized Trail

Time	0	10	25	30	31	40
%A	75	75	20	20	75	75
%B	15	15	80	80	15	15

Preparation of standard stock solution

Accurately weighed 100 mg of standard Rizatriptan was dissolved in 100 mL of mobile phase with proper sonication which gives strength of 1000 mcg/mL. This stock solution was filtered through 0.4 µ membrane filter paper.

Calibration curve for Rizatriptan

From the standard stock solution Rizatriptan respected aliquots are pipette out into 10mL volumetric flask and dilutions are made with mobile phase to obtain concentration range from 2-12µg/mL

Sample preparation

Drug equivalent to 1 mg of Rizatriptan was weighed and transferred into 10 mL volumetric flask. The drug was dissolved in 10 mL of mobile phase and sonicated for 5mins.

RESULTS AND DISCUSSION

Optimized chromatogram

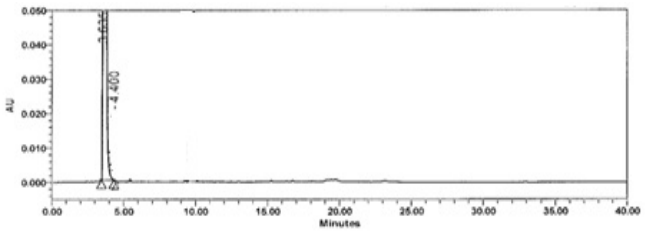

Fig. 2. Optimized Chromatogram of Rizatriptan

In Fig. 2, chromatogram represents Rizatriptan with an average retention time of 3.635 ± 0.126 min and with no interfering peaks which indicates the specificity of the HPLC method developed. The retention time was comparable with the shorter published data for Rizatriptan.

System Suitability

System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system.

Table 3. System Suitability results

Parameter	Result	Acceptance Limit
Retention time (Rt)*	Min	--
Resolution factor*	NA	--
Number of theoretical plates (N)*	2984	More than 2000
Tailing factor (T)*	1.54	Less than 2
* Number of injections: 6 replicates		

The system suitability was performed by 6 replicate analyses of Rizatriptan at a concentration of 8 µg/ml. The tailing factor as determined for Rizatriptan peak from standard solution was found to be 1.54. The theoretical plates were found to be 2984.The acceptance criterion for Number of theoretical plates (N) is more than 2000 and tailing factor is less than 2.

2. SPECIFICITY

Table 4. Specificity Data

S.No	Peak Name	
1	Blank	Nil
2	Placebo	Nil
3	Standard	R_t:3.635 min λ_{max} : 282 nm

No additional peak was detected close to the retention time of Rizatriptan which was found to be 3.635 min. whereas in the blank and placebo chromatograms no peak was detected at retention time of Rizatriptan from this it was concluded that this method is specific.

Intraday and interday precision

Table 5. Intraday and Interday Precision Results

S.No.	Intraday precision Area	Interday precision Area
1	205147	201478
2	205136	202147
3	208521	203512
4	208712	201485
5	208412	201569
6	201548	212542
Mean	206246	203788.8
Std Dev	2596.775	3978.388
%RSD	1.2	1.9

The optimized method was applied repetitively to analyze multiple replicates in three different occasions. Intraday precision was performed by analyzing of six replicates at a concentration of 8 µg/mL of standard Rizatriptan within the same day while the inter-day precision was performed by analyzing of six replicates at a concentration of 8 µg/mL of standard of Rizatriptan on three different days. The total precision of the method was expressed as the relative standard deviation (%RSD). The intraday and interday %RSD for Rizatriptan was 1.2 and 1.9 respectively meets the acceptance criteria of less than 2.0. %RSD

Linearity and range

Table 6. Linearity and range results

S. No	Concentration (µg/mL)	Peak Area
1	2	51331
2	4	102663
3	6	153993
4	8	205324
5	10	254587
6	12	307945

Linearity was determined by six different Rizatriptan standard concentrations in the range of 2-12 µg/mL. The average peak areas were plotted against concentrations and it was observed that the peak area is directly proportional to the concention of Rizatriptan. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation,slope and intercept. In general, a value of correlation coefficient (r2) > 0.998 is considered as the evidence of an acceptable fit for the data to the regression line. The Correlation coefficient of Rizatriptan was found to be 0.999.

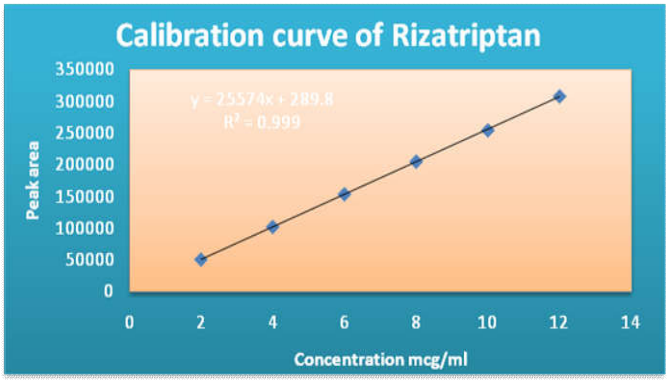

Fig. 3. Calibration curve of Rizatriptan

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%.

Table 7. Results of Accuracy

Spiked Concentration (µg/mL)	Peak area	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery	% Mean Recovery
4	205321	4.01	4.14	103.2419	101.7
	201478		4.09	101.995	
	203254		4.01	100	
8	201452	8.03	8.12	101.1208	100.4
	200958		8.07	100.4981	
	201254		8.02	99.87547	
12	212542	12.02	12.04	100.1664	100.1
	201562		12.06	100.3328	
	201356		12.01	99.91681	

The recovery studies were carried out 6 times and the percentage recovery was calculated. From the data obtained recoveries of Rizatriptan standard concentrations in the 4 µg/ml, 8 µg/ml, 12 µg/ml were found to be 101.7, 100.4, 100.1 respectively (Table 7). The method will be considered as accurate when the % recoveries were in between 98.0 and 102.0 according to ICH guidelines.

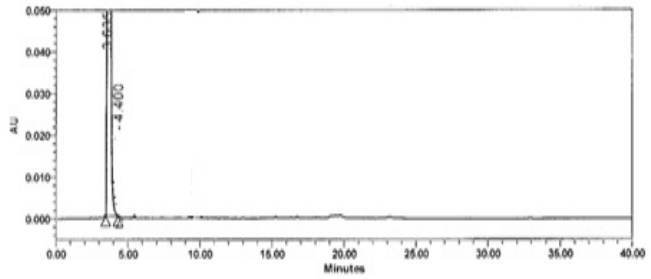

Fig.4. Chromatogram showing accuracy at 50% of standard Drug

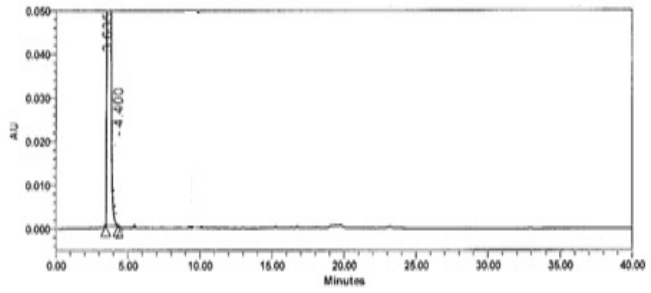

Fig.5. Chromatogram showing accuracy at 100% of standard Drug

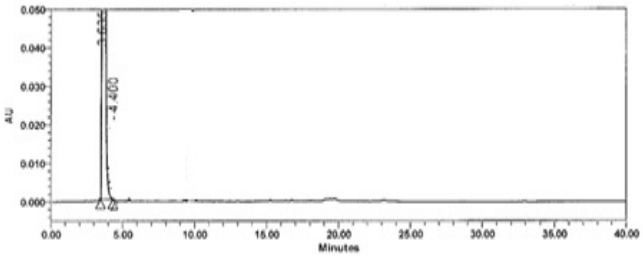

Fig.6. Chromatogram showing accuracy at 150% of standard drug

LOD & LOQ

Table 8. Results of LOD&LOQ

S.NO	Parameter	Slope	Standard Deviation	Value µg/mL
1	Limit of Detection	25574	2596.7	0.33
2	Limit of Quantification			1.01

LOD & LOQ are the least concentration of the drug that can be detected and quantified respectively. From LOD & LOQ the sensitivity of the method will be assessed. The LOD and LOQ were calculated as LOD = 3.3 σ/S and LOQ = 10 σ/S Where r is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. The LOD & LOQ was found to be 0.33 and 1.01 respectively.

Robustness

Table 9. Change of Flow rate (± 10%)

S.No	Flow Rate	0.9mL/min	1mL/min	1.1mL/min
1		201425	204758	206958
2		202536	205869	205847
3		210211	211109	211201
4	Mean	204724	207245.3	208002
5	Std dev	3906.315997	2769.419	2307.059
6	% RSD	1.91	1.33	1.11

Table 10. Change in Temperature (± 5°C)

S.No	Temperature	30 °C	35 °C	40 °C
1		203214	206547	203698
2		206547	209874	202587
3		210215	201987	211458
4	Mean	206658.6667	206136	205914.3
5	Std dev	2859.236767	3232.943	3946.117
6	% RSD	1.38	1.56	1.91

The robustness of the method was studied by deliberate changes in the method like alteration in flow rate and temperature. The robustness studies of Rizatriptan samples were passed the acceptance criteria of less than 2% RSD which indicated that that there were no marked changes in the chromatograms and demonstrate that the HPLC method developed was robust.

Assay: The assay of Rizatriptan bulk drug was found to be 100.73 %

Conclusion

Selective and precise RP-HPLC method was developed for estimation of Rizatriptan in pure form. Retention time of Rizatriptan was 3.635 min which reduces the analysis time. The method underwent various validation parameters and the results were below the acceptance criteria. So the method can

be used for the routine analysis of Rizatriptan pure form without any interference of Excepients.

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