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Method development and validation of rizatriptan in dosage form by RP-HPLC

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ABSTRACT

Rizatriptan is a drug used in the treatment of migrane. This attempt was made to develop a simple, accurate & precise method for the routine analysis of Rizatriptan. This method was developed on trial & error basis by changing the variables wherever required. Finally a method was optimized and the conditions were determined by using RP HPLC Method. The optimized method used contains 70 volumes of phosphate buffer P^h7.8 and 30 volumes of acetonitrile at 235 nm and is validated as per ICH guidelines. The method was validated for system suitability, linearity, precision, accuracy, specificity, robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration ranges of 40-70 µg / ml for Rizatriptan and R^2 value was found to be 0.998. As there was no interference due to mobile phase, the method was found to be specific. The method was also found to be robust and rugged. The proposed method developed was suitable for the quantitative determination of Rizatriptan in dosageforms.

Keywords: Rizatriptan, RP-HPLC, Method Development, Validation

INTRODUCTION [1]

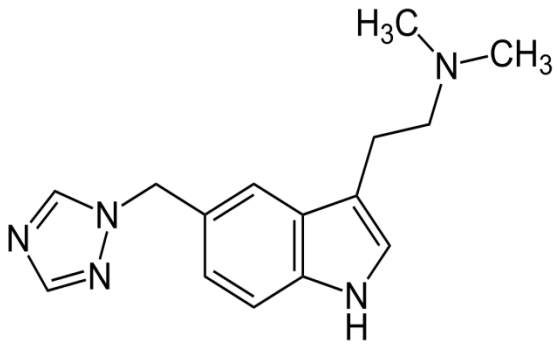
High Performance Liquid Chromatography

HPLC is a chromatographic technique which is used to separate a mixture of components into individual compounds. This technique is most commonly used in biochemistry and in analytical chemistry to identify, quantify and purify the substances.

Rizatriptan [9]

Rizatriptan, is a triptan drug which used in the treatment of migraine. It belongs to the class of organic compounds known as tryptamines and its derivatives. These compounds contain tryptamine

backbone, which is structurally characterized by an indole ring subsituted at the 3-position by an ethanamine.



Rizatriptan is chemically, dimethyl({2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethyl})amine. It is fine white powder and freely Soluble in Methanol, Acetonitrile and Acetone. Several methods have been developed for determination of Rizatriptan in pharmaceutical preparation by UV Spectrophotometry, HPTLC and HPLC [10]. The proposed methods were validated in accordance with USP and as per ICH guidelines.

MATERIALS AND METHODS [2, 3]

Materials

Rizatriptan was procured as a gift sample from Chandra Labs, Hyderabad, India. HPLC grade Acetonitrile (HPLC Grade) and Milli-Q water was used.

Instruments

HPLC (Shimadzu (LC 20 AT VP) Software: Spin chrome (LC SOLUTIONS), UV detector), Global digital pH meter and Digital weighing balance (MettlerToledo), Volumetric flask, Pipettes and burettes, Beakers (Borosil), Digital ultra sonicator (Fast clean).

HPLC METHOD DEVELOPMENT

Preparation of mobile phase

The mobile phase used was a mixture of acetonitrile and phosphate buffer (pH 7.8) in the ratio of 70:30 v/v; it was filtered before use through a 0.45 µm membrane filter and degassed for 30 min. The elution was carried out isocratically at the flow rate of 1.0 ml/min. Detection was carried out at 232 nm at ambient temperature.

Diluent preparation: The mobile phase is used as diluent.

Preparation of buffer

2.72gm of potassium dihydrogen phosphate (KH_2PO_4) was weighed and dissolved in 100ml of water and volume was made up to 1000ml with water. Adjust the pH to 3.5 using orthophosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of standard solution

Accurately weigh and transfer 10 mcg of Rizatriptan working standard into a 10ml clean, dry volumetric flask and add about 5ml of diluent. Sonicate to dissolve it completely and make up the volume up to the mark with the same solvent (stock solution)

Preparation of sample solution

Weigh and transfer 10µg of sample into a 10 ml volumetric flask. Dissolve in 5ml of diluent and make up the volume.

Optimized Chromatographic Conditions

Instrument used: Shimadzu HPLC with auto sampler and UV detector
Temperature: Ambient
Column: Inertsil ODS 3V(250x4.6mm) 5µm
Mobile phase: Acetonitrile : Phosphate buffer
Flow rate: 1.0 ml/min

Wavelength:235nm, 225nm.
Injection volume: 20µl
Run time: 15min.

VALIDATION PARAMETERS [4, 5]

System Suitability

From the standard stock solution, pipette out 1.0 ml solution into a 10ml volumetric flask and dilute up to the mark with the diluents. The standard solution was injected for six times, peak area and retention times were measured for all the injections in HPLC. The % RSD for the retention times and peak area of RIZATRIPTAN was found to be less than 2%.

Specificity of the Drug by Direct comparison method

Preparation of Standard Solution

Weigh accurately 10 µg of RIZATRIPTAN and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 50 µg/ml of RIZATRIPTAN is prepared by diluting 1ml to 50ml with mobile phase. This solution is used for recording chromatogram.

Preparation of Sample

10 Tablets (each tablet contains 10 µg of RIZATRIPTAN) were weighed and finely powdered. Test stock solutions of RIZATRIPTAN (50µg/ml) were prepared by dissolving weight equivalent to 50 µg of RIZATRIPTAN and dissolved in sufficient mobile phase. The solution was later on filtered using 0.45-micron syringe filter and Sonicated for 5 min and the volume is made upto 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 50 µg/ml of RIZATRIPTAN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Linearity

From the standard stock solution aliquots of 30, 40, 50, 60, 70 µg/ml are prepared and inject each level into chromatographic system and determine the peak area.

Plot a graph of peak area v/s concentration (X axis- concentration and Y axis- peak area) and calculate the correlation coefficient.

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%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug. The percentage mean recovery of RIZATRIPTAN was found to be 99.45%.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guideline.

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

Where σ = the standard deviation of the response and S = the standard deviation of y-intercept of regression lines.

Precision

Method Precision

Prepared sample preparations of RIZATRIPTAN as per test method and injected 6 times in to the column.

Robustness

Chromatographic conditions variation

Robustness of the method is determined by injecting the prepared solutions at different variable conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analyst

RESULTS AND DISCUSSION

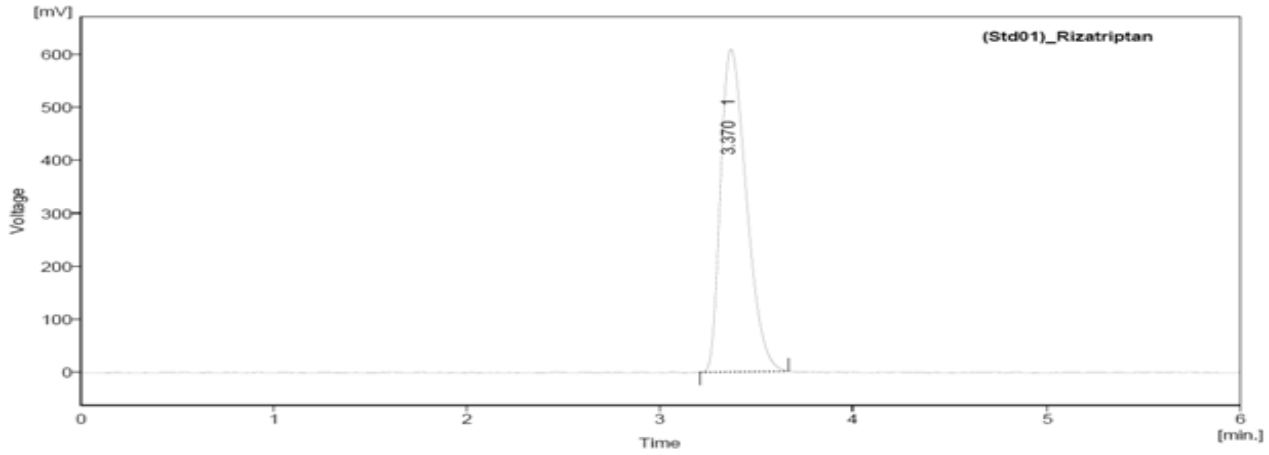


Figure-1: Optimized Chromatogram For Standard

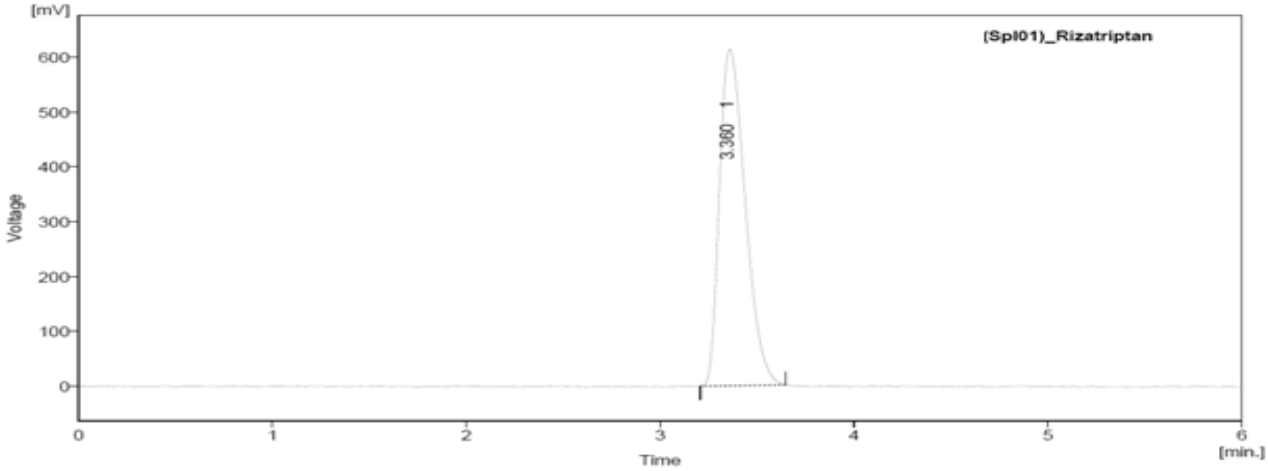


Figure-2: Optimized Chromatogram of Sample

Table-1: Peak Results For Optimized Chromatogram (Standard & Sample)

Peak name	R_t	Area	Height
STD	3.370	5669952	60910
SAM	3.360	5671137	61376

VALIDATION

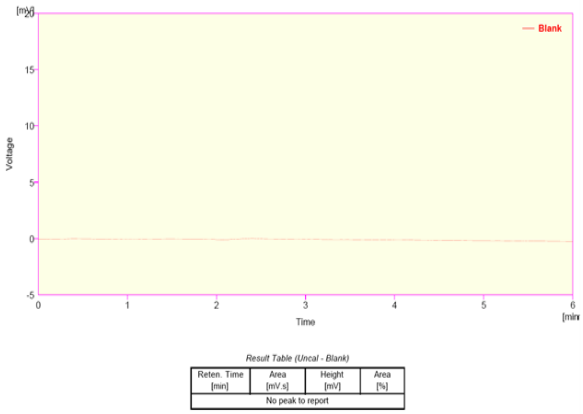
System Suitability

Table-2: Data of System Suitability.

Injection	Retention time (min)	Peak area
1	3.383	5655.120
2	3.377	5681.870

3	3.377	5672.110
4	3.373	5668.474
5	3.370	5695.459
6	3.373	5667.884
Mean	3.3755	5673.486
SD	0.0045	13.775
%RSD	**0.13**	**0.24**

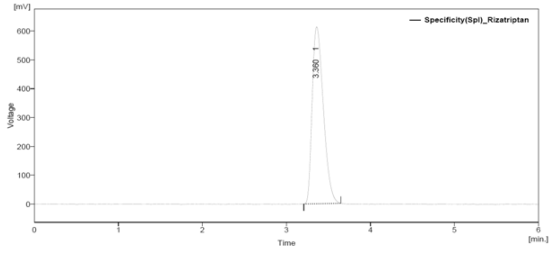
Specificity



Result Table (Uncal - Blank)

Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
No peak to report			

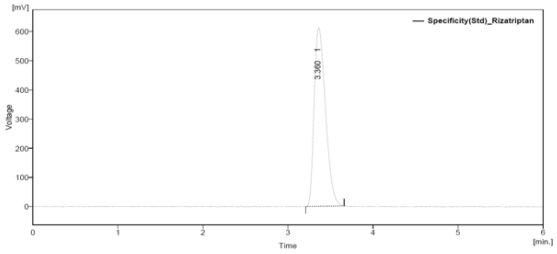
Figure-3: Blank Chromatogram for Specificity by Using Mobile Phase



Result Table (Uncal - Specificity(Spl)_Rizatriptan)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.360	5671.137	613.762	100.0	100.0	0.15
	Total	5671.137	613.762	100.0	100.0	

Figure-4: Chromatogram For Specificity Of RIZATRIPTAN Sample



Result Table (Uncal - Specificity(Std)_Rizatriptan)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.360	5662.791	612.488	100.0	100.0	0.15
	Total	5662.791	612.488	100.0	100.0	

Figure-5: Chromatogram For Specificity Of RIZATRIPTAN Standard

Linearity

Coefficient correlation was found to be 0.998

Table-3: Chromatographic Data For Linearity

S.No.	Conc.(µg/ml)	Area
1	30	3658.699
2	40	4687.349
3	50	5665.736
4	60	6861.637
5	70	7798.355

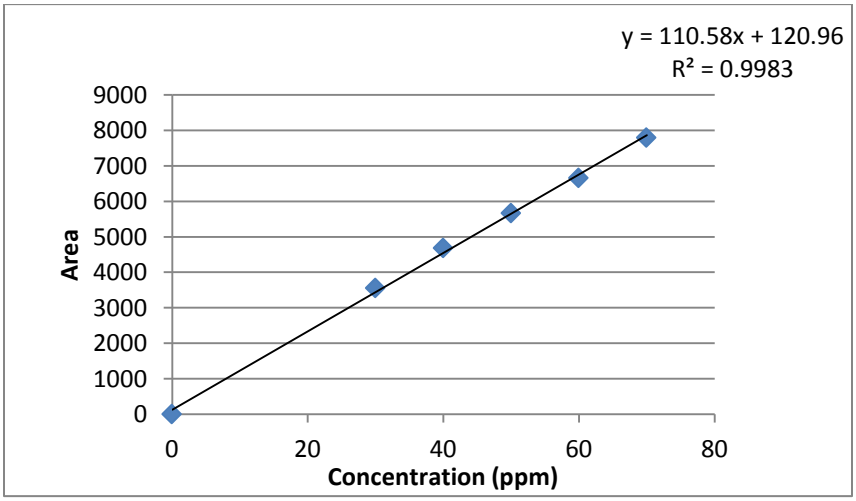


Figure-6: Linearity Curve For Rizatriptan

Table-4: Data of Accuracy for concentrations 50%, 100%, 150%

Recovery level	Accuracy RIZATRIPTAN					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50%	50	**5658.261**				
	50	**5658.261**	**5660.485**	**49.25**	**98.50**	
	50	**5664.934**				
100%	60	**6861.637**				
	60	**6913.117**	**6891.517**	**60.82**	**101.36**	99.45
	60	**6899.797**				
150%	70	**7438.255**				
	70	**7414.595**	**7426.410**	**68.96**	**98.51**	
	70	**7426.380**				

Limit of Detection

The limit of detection of an individual analytical procedure is the lowest quantity of analyte in a sample which can be detected but not essentially quantitated. The LOD is found to be 0.56 µg/ml

Limit of Quantification

The limit of quantification of an individual analytical process is the lowest quantity of analyte in a sample which can be quantitatively determined.

The limit of quantification was found to be 1.69 µg/ml

Precision

The % RSD of sample preparations of RIZATRIPTAN was found to be less than 2.0%.

Table-5: Data of Precision

	RIZATRIPTAN	
S.No.	**Rt**	**Area**
1	3.383	5655.120
2	3.377	5681.870
3	3.377	5672.110
4	3.373	5668.474
5	3.370	5695.459
6	3.373	5667.884
Avg	3.3755	5673.486
Stdev	0.0045	13.775
%RSD	**0.13**	**0.24**

Robustness

System suitability parameters were found to be within limits at all variable conditions.

Table-6: Results of Robustness

Parameter	**RIZATRIPTAN**	
	Retention time(min)	**Tailing factor**
Flow		
1.0ml/min	4.94	1.914
1.4ml/min	2.957	1.731
Wavelength		
237nm	3.423	1.767
241nm	3.417	1.767

Ruggedness

%RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table-7: Results of Ruggedness

Parameter	RIZATRIPTAN	
	Retention time(min)	**Tailing factor**
Flow		
1.0ml/min	4.94	1.914
1.4ml/min	2.957	1.731
Wavelength		
237nm	3.423	1.767
241nm	3.417	1.767

CONCLUSION

In this attempt a simple, accurate & precise method for the routine analysis of Rizatriptan was developed on trial & error basis by changing the variables wherever required. Finally a method was optimized and the conditions were determined by using RP HPLC. The method was validated for system suitability, linearity, precision, accuracy, specificity, robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The results obtained are expressed in tables above.

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