Life Cycle Management of Analytical RP-HPLC Method Development for Assay of Rizatriptan in Immediate Release Dosage Form

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Abstract:
In this research work of life cycle management of analytical RP-HPLC method development for assay of Rizatriptan in immediate release dosage form, the RP-HPLC assay method was developed and validated for Rizatriptan. Stress study is also carried out. The chromatographic conditions will be as, Symmetry C-18, 150 mm x 4.6 mm, 5 µm column at 30°C temperature by using a mobile phase [Mixture of 100 mL Acetonitrile, and 900 mL Buffer solution] at a flow rate of 1.8mL/min, and UV detection at 225 nm and run time is 15 min. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Rizatriptan in immediate release dosage form.

***Key words*: Rizatriptan, Life Cycle Management, RP-HPLC Method Development, Validation, Immediate Release Dosage Form**

Materials and Methods:

Introduction:

Rizatriptan benzoate is N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine. It is an anti migraine drug, which selectively activates 5-HT1B/1D receptors. Physical properties are white to off white crystalline powder, soluble in water, melting point 178–180_, and stable under ordinary condition. The some published methods of analysis for determination and separation of Rizatriptan in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Rizatriptan. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Rizatriptan in immediate release dosage form.

Structure of Rizatriptan benzoate

Experimental:

Materials:

1) Risatriptan (Rizatriptan Benzoate): - Working standard and its claimed purity was 98.20%.
2) Risatriptan (Rizatriptan Benzoate) Tablet (label claim 5 and 10 mg) and placebo, which was prepared and supplied by Instavision lab.

- Reagents and Chemicals:
  1) Acetonitrile: -HPLC grade, Rankem, India.
  2) Methanol: - HPLC grade, Rankem, India.
  3) Milli-Q water: - It was purified by Millipore Corporation's system.
  4) Acetic acid: - Reagent Grade, Merck, India.
  5) Hydrochloric acid: - Reagent Grade, Merck, India.
  6) Sodium hydroxide: - Reagent Grade, Merck, India.
  7) Hydrogen Peroxide (30%):- Reagent Grade, Merck, India.
  8) Sodium Perchlorate :- Reagent Grade, Merck, India.
  9) Triethylamine :- Reagent Grade, Merck, India.

Instruments, Apparatus and equipment:

1) High Performance Liquid chromatography system (HPLC): Agilent Liquid Chromatography with PDA detector
2) Chromatographic software:– E Z Crome Elite
3) A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - UV- 2450, Shimadzu, Japan.

4) Analytical Balance: - AD 265S, Mettler Toledo, Sweetzerland.
5) pH Meter: - Labindia, India.
6) Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
7) Hot air oven: - Labline, India.
8) Photo stability chamber: - SVI equipments, Germany

➢ Chromatographic system:

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1322A), binary pump (G1312A), auto injector (G1367C), column oven (G1310B), DAD detector (G1315C) and E Z Crome Elite (software).

The published methods of analysis for determination and separation of Rizatriptan in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Rizatriptan.

➢ Selection of Buffer in Mobile Phase: -

Sodium perchlorate and 1ml Triethylamine in 1000ml water and adjusted pH 2.00, 3.00, 4.00 and 5.00 with 10% Acetic acid was used to optimize the peak shape and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (90:10) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

➢ Selection of Mobile Phase: -

Acetonitrile was used to optimize the retention time of late eluting impurities and Methanol to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (90:10) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

➢ Selection of Column:-

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of degradation products and impurities, a reversed phase C_{18} column was preferred over other columns to separate all polar impurities as Symmetry C-18, 150 mm x 4.6 mm, 5 µm column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

➢ Selection of Diluent / Solvent for extraction:-

Different solvents were tried including single solvent and combination of solvents like Acetonitrile and methanol in different concentrations, But Risatriptan (Rizatriptan Benzoate) tablet gets dissolved in Solvent Mixture: [Bufferl: Acetonitrile 90:10] and hence mobile phase is used as diluent

Various Method screening Trials has been taken using following different compositions.

➢ Table for trials:

Sr.No.	Trails Taken	Observation	Remarks
1	Buffer pH2.00 : Methanol (70:50 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	No Peak observed	Not Satisfactory
2	Buffer4.00 : ACN (70:50 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	No Peak observed	Not Satisfactory
3	Buffer: Acetonitrile (70:30 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Peak observed	Not Satisfactory
4	Buffer: Acetonitrile : Methanol (80:10:10 v/v), Flow rate 2.0 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Broaden peak observed	Not Satisfactory
5	Buffer: Acetonitrile : Methanol (90:05:05 v/v), Flow rate 1.8 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Tailing observed	Not Satisfactory
6	Acetonitrile : Buffer (10:90 v/v), Flow rate 1.8 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Good peak shape observed and separated from benzoic acid peak	Satisfactory

Reason for validation: Non-Pharmacopeial method.

Design of experiment (DOE):

A smart DOE was performed with respect to components of mobile phase (like concentration of buffering agent/ buffer strength, pH of buffer, ratio of buffer ad organic modifiers) and chromatographic parameter (like Flow rate and column temperature) as mentioned below.

1. Molarity of buffer Sodium Perchlorate conc. 1gm +/- 0.01g
2. Triethylamine conc. 1mL +/- 0.01mL
3. pH of buffer pH 4.3 +/- 0.2
4. Buffer ratio 900 mL +/- 90mL

5. Acetonitrile 100 mL +/- 10mL
6. Flow rate 1.8 +/-0.2mL
7. Column temp 30+/- 5 °C

Method Validation:

- Standard preparation:

Weigh and transfer about 100 mg of Risatriptan (Rizatriptan Benzoate) reference standard to a 100 mL volumetric flask and dissolve and dilute up to the mark with mobile phase, further dilute 10mL solution to 100mL with mobile phase.

- Sample preparation

Weigh accurately not less than 20 tablet crush and weigh powder equivalent to 100mg of label amount into 100 mL volumetric flask add about 75 mL of mobile phase, sonicate at for about 15 min with intermittent shaking, keep achieve room temperature make up to volume with mobile phase, further dilute 5mL solution to 50mL with mobile phase.

- Buffer Preparation

Added 1 gm of sodium perchlorate and 1ml triethylamine in 1000ml water and adjust pH 4.30 with 10% Acetic acid.

- Mobile phase Preparation

Mix 100 ml of Acetonitrile and 900ml of buffer solution, sonicate and filter through 0.45µ membrane filter and degas.

- Diluent/Blank Solution:
  Use mobile phase as blank.
- Optimized HPLC Parameters:

Instrument	: Agilent Liquid Chromatography with PDA detector
Column	: Symmetry C-18, 150 mm x 4.6 mm, 5.0 µm
Flow Rate	: 1.8 mL/min
Injection volume	: 20 µL
Column temperature	: 30°C
Sample cooler Temperature	: Ambient
Detection	: 225 nm
Run time	: 15 minutes

System Suitability Test:

Sr. No.	Parameters	Risatriptan (Rizatriptan Benzoate)
1.	Peak area	*5670421*
2.	No. of theoretical plates	*8529*
3.	Retention time (min)	5.312
4.	Asymmetry/USP Tailing	1.02
5.	% RSD	*0.11*

Specificity:

Specificity Part-I: Interference from blank, benzoic acid and placebo

- Procedure

Prepare blank preparation, prepared placebo preparation, standard preparation, and sample preparation for 5 and 10mg tablet as per the method.

- Benzoic acid solution preparation

Transfer accurately measured quantity of acetic acid 50 mg and transferred to a 100 mL volumetric flask add about 75 mL of mobile phase , mix and make up to volume with mobile phase, further dilute 10mL to 100mL with mobile phase.

- Placebo preparation

Weighed accurately placebo equivalent to 100 mg of Risatriptan (Rizatriptan Benzoate) and transferred to a 100 mL volumetric flask add about 75 mL of mobile phase , sonicate at for about 15 min with intermittent shaking, keep to achieve room temperature make up to volume with mobile phase, further dilute 10mL to 100mL with mobile phase.

Observation: No interference seen

Specificity Part-II :Forced degradation

Sr. No.	Stress type	% Degradation	Observation
1	Untreated sample	----	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate).
2	Heat degradation (Solid state)	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
3	Heat degradation (Solution state)	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
4	Photolytic degradation	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
5	Humidity degradation	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
6	Acid dégradation	5.86%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
7	Base degradation	10.15%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)

Sr. No.	Stress type	% Degradation	Observation
8	Peroxide degradations	16.53%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)

Linearity and Range:

Linearity Level	Standard concentration	Concentration of Risatriptan (Rizatriptan Benzoate) (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	50.20	2952527	0.9997
Level – 2	80%	75.30	4051177	
Level – 3	100%	100.40	5405053	
Level – 4	120%	125.50	6390534	
Level – 5	150%	150.60	7457580	

Precision:

Sample Preparation	% Assay of Risatriptan (Rizatriptan Benzoate)
Test solution -1	99.67
Test solution -2	99.59
Test solution -3	99.55
Test solution -4	99.38
Test solution -5	99.48
Test solution -6	99.69
Mean	99.56
Standard Deviation	0.12
Relative Standard Deviation (%)	0.12

Intermediate precision:

Analysis performed during method precision study Analyst: Analyst-I HPLC ID No.: EAR040 Make :Symmetry,C18, 4.6mmx150mm, 5 µm Column serial number. : 0402471K	
Sr. No.	% Assay of Risatriptan (Rizatriptan Benzoate)
Test solution-1	99.67
Test solution-2	99.59
Test solution-3	99.55
Test solution-4	99.38
Test solution-5	99.48
Test solution-6	99.69
Analysis performed during intermediate precision study HPLC ID No.: EAR039 Make :Symmetry,C18, 4.6mmx150mm, 5 µm Column serial number : 0502481L	
Test solution-1	99.44
Test solution-2	99.56
Test solution-3	100.05
Test solution-4	99.42
Test solution-5	99.64
Test solution-6	99.83
Mean of twelve samples	99.66
Standard Deviation	0.24
Relative Standard Deviation (%)	0.24

Robustness:

- Change the flow rate of Mobile Phase:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in flow rate 1.6 mL/ min.	1	99.65
	2	99.38
Mean		99.55
Standard deviation		0.12
Relative standard deviation (%)		0.12
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in flow rate 2.0 mL/ min.	1	99.21
	2	98.99
Mean		99.44
Standard deviation		0.24
Relative standard deviation (%)		0.24

- Change in the Mobile Phase composition ±10%:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in organic component +10%	1	99.78
	2	99.52
Mean		99.55
Standard deviation		0.13
Relative standard deviation (%)		0.13
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59

	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in organic component -10%	1	99.11
	2	99.68
Mean		99.52
Standard deviation		0.20
Relative standard deviation (%)		0.20

- Change in the Temperature of the Column ±5°C:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Temperature of Column oven +5°C	1	*99.05*
	2	*100.65*
Mean		*99.63*
Standard deviation		*0.46*
Relative standard deviation (%)		*0.46*
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Temperature of Column oven -5°C	1	*101.01*
	2	*100.65*
Mean		*99.88*
Standard deviation		*0.60*
Relative standard deviation (%)		*0.60*

- Change in buffer component of mobile phase ±10%:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*

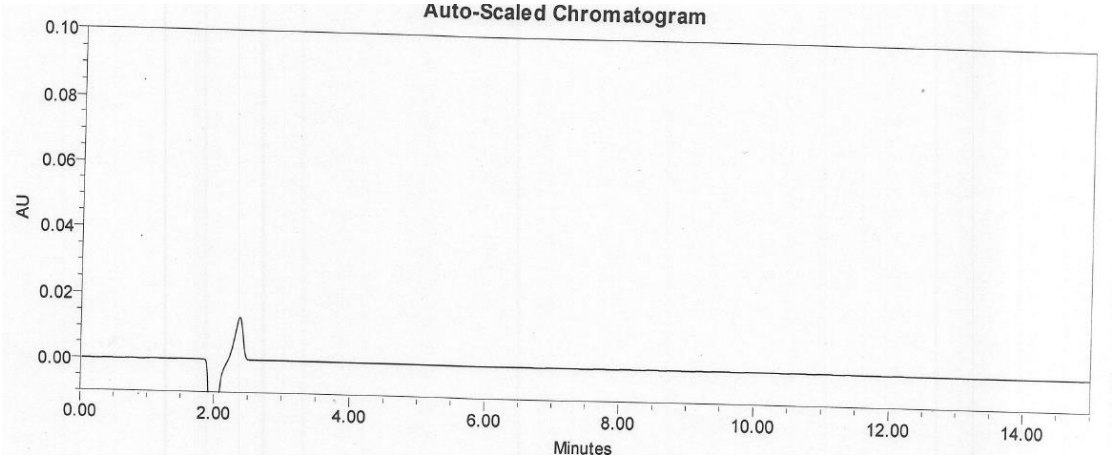
	5	*99.48*
	6	*99.69*
Change in Buffer component +10%	1	*100.25*
	2	*100.01*
Mean		*99.70*
Standard deviation		*0.29*
Relative standard deviation (%)		*0.29*
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Buffer component -10%	1	*99.65*
	2	*100.15*
Mean		*99.65*
Standard deviation		*0.23*
Relative standard deviation (%)		*0.23*

- System suitability parameters:

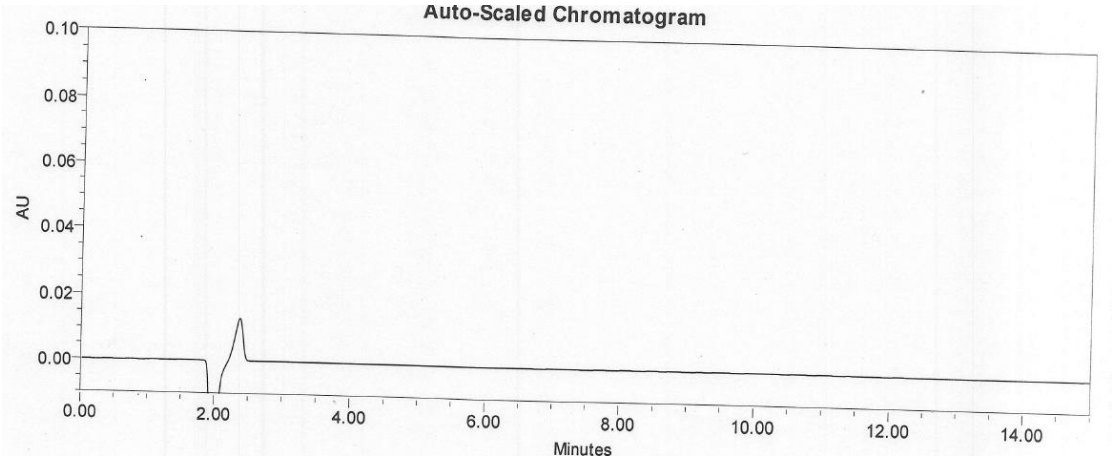
Parameter			Theoretical Plates	Tailing Factor	%RSD
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	Specificity				
	1.1	Specificity-Part-A	8529	1.02	0.11
	1.2	Specificity-Part-B	8313	1.05	0.15
2	Linearity and Range		8204	1.02	0.18
3	Accuracy study (Recovery)		8735	1.02	0.23
4	Precision				
	4.1	Method precision (Repeatability)	8526	1.04	0.12
	4.2	Intermediate Precision (Ruggedness)	8431	1.06	0.24
5	Robustness				
	5.1	Change flow rate by ± 10% (1.6 ml/minute and 2.0 ml/minute).	7952 8358	1.09 1.24	0.12 0.47
	5.2	Change the column temperature by ± 5°C (25°C and 35°C)	7952 8878	1.08 1.01	0.62 0.19
	5.3	Change the mobile phase Organic components by ± 10%	7952 6754	1.09 1.11	0.58 0.39
	5.4	Change the mobile phase Buffer components by ± 10%	7952 8886	1.09 1.31	0.58 0.79

Reason for validation: Non-Pharmacopeial method.

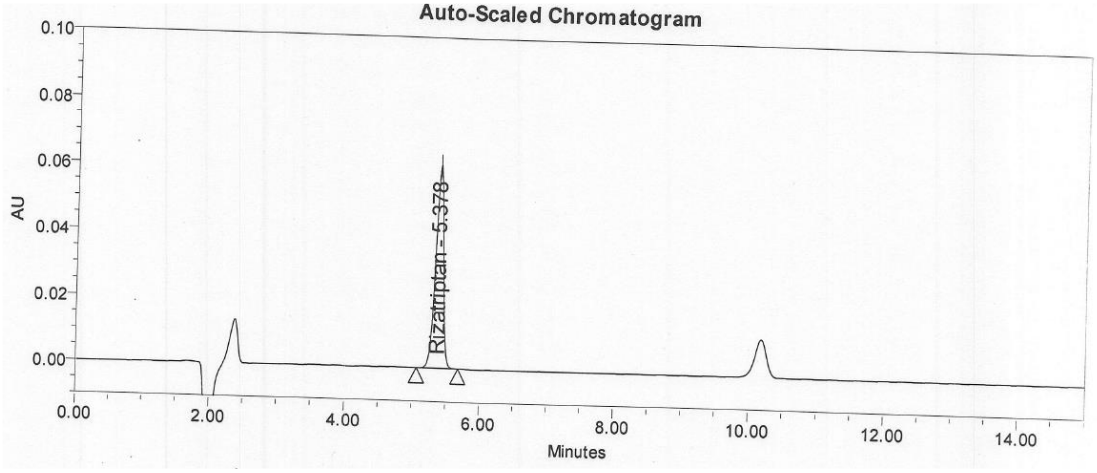
The results of analysis in terms of % label claim were found to be 98.00 to 102.00 for formulation analyzed.
Chromatograms:



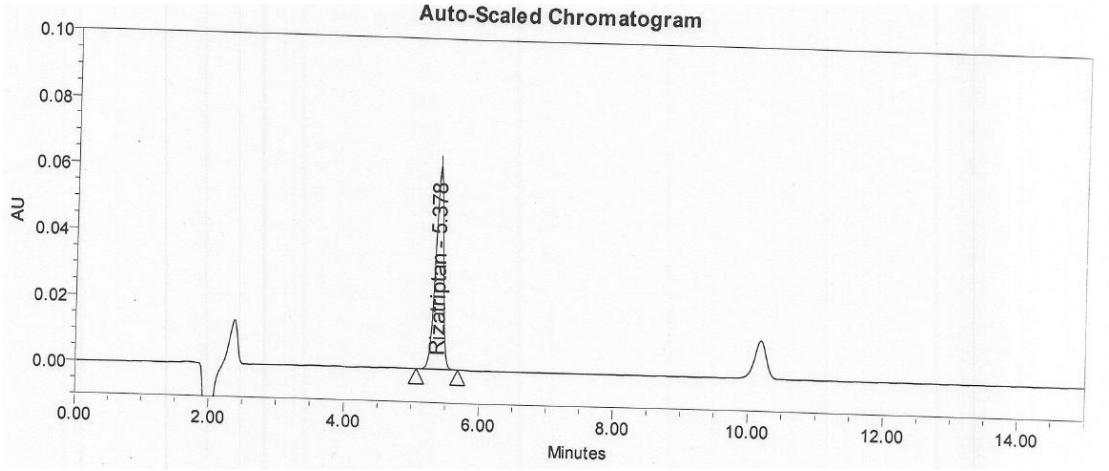
Blank



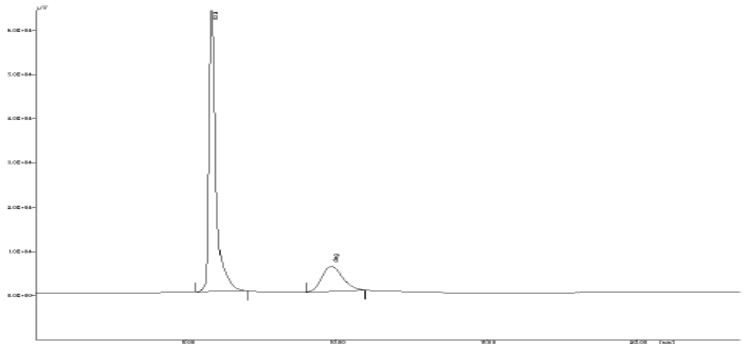
Placebo



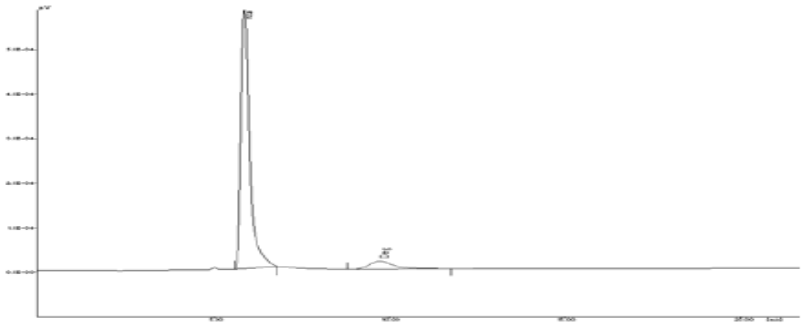
Standard



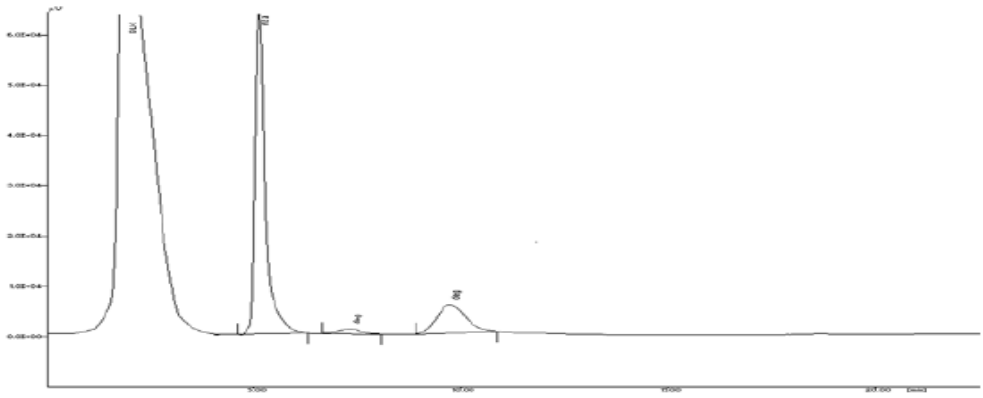
Sample



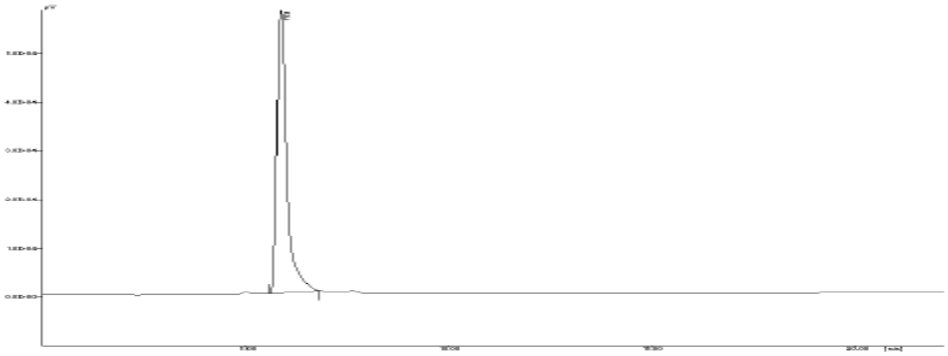
Chromatogram of Base degradation sample for Risatriptan (Rizatriptan Benzoate) tablet



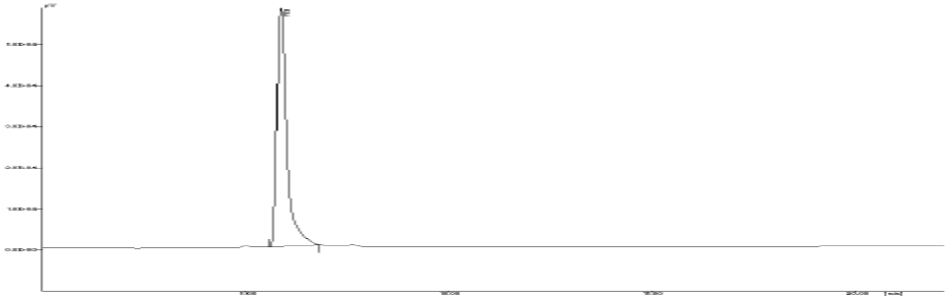
Chromatogram of Acid degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



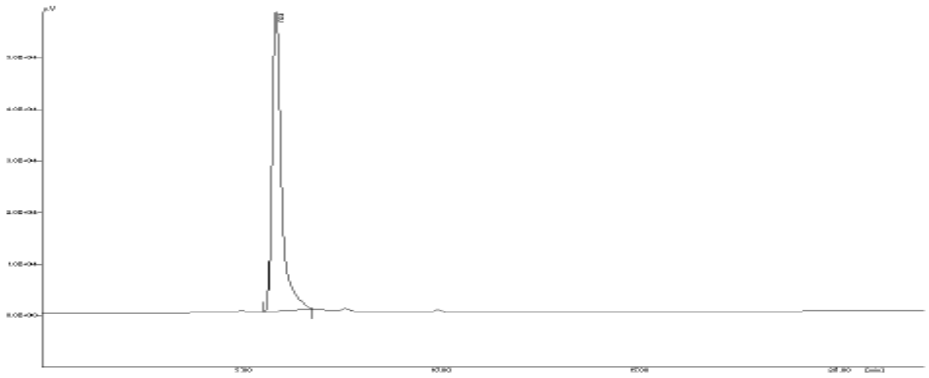
Chromatograms of per oxidation degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of thermal-degraded-Solution state sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of photo-degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of Humidity degradation sample for Risatriptan (Rizatriptan Benzoate) tablet

Result and Discussion:

- Specificity Part-I

There is no interference of blank and placebo peaks with the main peak. All impurities are well separated from the main peak. The main peak purity and known impurities purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method can be termed as specific.

- Specificity Part-II

- Degraded impurities in all sample preparation are well separated from the main peak.
- Peak purity for the main peak in sample preparation is well within the limit of acceptance criteria.

Hence the method can be termed as specific

- Linearity and Range

The areas obtained are directly proportional to the concentration of analyte in the sample. Hence the method considered as linear in the range considered.

- Accuracy

The recovery at each level and mean recovery meets the established acceptance criteria.
Hence, the method can be termed as accurate in the considered range.

- Precision

The results obtained lie well within the limit of acceptance criteria. Hence the method can be termed as precise and rugged.

- Filter media interference

The results obtained lie well within the limit of acceptance criteria. Hence there is no interference from filter media.

- Robustness

No significant changes observed in system suitability parameters.
Hence, the method can be termed as robust.

- System Suitability

The mean values of system suitability parameters are well within acceptance criteria, hence the method is suitable
Since the results are within the limit of acceptance criteria for all validation parameters, therefore, the method can be considered as validated and suitable for intended use.

Conclusion:

The proposed method for determination of Risatriptan (Rizatriptan Benzoate) is simple, specific, rapid, linear, accurate, precise, rugged, robust, sensitive as well as selective and suitable for routine analysis in laboratories.

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References:

1. Avinash M. Bhagwat, Anand P. Khadke, Anuradha M. Patil, Snchal V. Tarade. Quality preparation work: the basic need of analysis. Safe handling of laboratory. European Journal of biomedical and pharmaceutical science. 2017.volume:4.Issues: 4. Page no: 158-165

2. Chandrashekhar K. Gadewar , Yogendrakumar Sahu, A.V. Chandewar, Pankaj Baghel, Devendra Kushwaha, Stability indicating method development and validation of assay method for the estimation of rizatriptan benzoate in tablet, Arabian Journal of Chemistry (2017) 10, S2067–S2072.

3. M.Peris: An overview of recent expert system applications in analytical chemistry Crit. Rev. Anal. Chem 1996; 26:219-237.

4. A.B. Roge, P.S. Tarte, M.M. Kumare, G.R. Shendarkar, S.M. Vadvalkar. Forced Degradation Study: An Important Tool in Drug Development. Asian J. Pharm. Res. 3(4): Oct. - Dec.2013; Page 198-201.

5. Soumesh Kumar Tripathy. Pharmaceutical Validation: A Quality Maintaining Tool for Pharmaceutical Industry. Asian J. Pharm. Res. 2020; 10(4):307-311.

6. Abdul Saleem Mohammad, Swetha Devidi, Nikhat Fatima, Humera Badar, Syeda Saba Sulthana, Mohammad Akthar Sulthana, Nuha Rasheed. An Overview of Validation and Basic Concepts of Process Validation: Quality Assurance View Point. Asian J. Pharm. Tech. 2016; 6 (3): 169-176.

7. Rajesh Z. Mujoriya. Analytical Method Development and Validation of Pharmaceutical Technology: An Overview . Research J. Pharma. Dosage Forms and Tech. 2013; 5(4): 213-220.

8. Prajkta M. Ghagare, Ashwini R. Patil, Bhavna J. Deshmane, Manish S. Kondawar. Review on Pharmaceutical Validation. Res. J. Pharma. Dosage Forms and Tech.2020; 12(1): 17-26.

9. Vishakha Shingote, S. D. Mankar, S. B. Dighe. A Review Article on Analytical Methods Development and Validation. Research Journal of Science and Technology. 2022; 14(1):77-3.

10. Chavan Pooja Ajit, Shelar Reshma Dattatraya, Shelake Pallavi Ramchandra, Avinash Mahadeo Bhagwat, Dr. Ajit Bhiva Ekal, RP-HPLC Method Development and Validation of Tadalafil in Tablet Dosage form, Asian J. Research Chem. 14(5): 1-9.