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Integrated Analytical Quality by Design (AQBD) Approach for the Development and Validation of Liquid Chromatography Method for Simultaneous Estimation of Rimantadine

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A B S T R A C T

A simple, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of Rimantadine in bulk and pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines. Method optimization confirmed that flow rate, buffer pH, and organic ratio significantly influenced chromatographic performance. The optimized method employed an Inspire C18 column (3.0 × 150 mm, 5 µm) with a mobile phase consisting of phosphate buffer (pH 3.0) and methanol, delivered at a flow rate of 1.0 mL/min, with detection at 265 nm. A sharp and symmetrical peak was obtained at a retention time of 6.12 min, with system suitability parameters meeting all acceptance limits (plate count >5800, tailing factor ~1.0).Validation studies demonstrated excellent linearity in the concentration range of 15–75 µg/mL (R^2=0.9994), high precision with %RSD values below 0.2%, and accuracy with mean recoveries between 96.7–101.2%. The method was sensitive with LOD and LOQ values of 2.96µg/mL and 9.97µg/mL, respectively. Robustness testing confirmed that deliberate variations in flow rate and mobile phase composition did not significantly affect results. Overall, the developed RP-HPLC method is accurate, reproducible, cost-effective, and stability-indicating, making it highly suitable for routine quality control, batch release, and regulatory applications of Rimantadine formulations.

Keywords: Rimantadine, RP-HPLC, Method Validation, ICH Q2(R1), Stability-indicating, Quality Control.

A R T I C L E I N F O

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Contents

1. Introduction

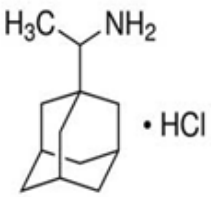

Fig.1: Rimantadine

Table.1: Rimantadine drug profile

Molecular Formula	$C_{12}H_{21}N$
Molecular Weight	11165.62 Da
IUPAC Name	1-(adamantan-1-yl)ethan-1-amine
Chem Spider ID	4893
Refractive Index	54.52 $m^3 \cdot mol^{-1}$
Polar Surface Area	26.02 $Å^2$
Log P	3.28 (Octanol/Water)
Generic Name	Rimantadine

Brand Names	Flumadine
Drug category	Aadmantane Antivirals, Antiviral Drug
Indications	Prophylaxis and treatment of influenza A virus infection.
Pharmacology	Inhibits viral replication by blocking proton transport across the M2channel.
Potency	Highly potent against influenza A virus
Tolerability	Generally well tolerated.
Contraindications	Hypersensitivity to rimantadine or other
Adverse Effects	Insomnia, headache, dizziness, nausea
Availability	Available in oral form.
Mechanism of Action	The mechanism of action of rimantadine is not fully understood. Rimantadine appears to exert its inhibitory effect early in the viral replicative cycle, possibly inhibiting the uncoating of the virus. The protein coded by the M2 gene of influenza A may play an important role in rimantadine susceptibility.

2. Materials and Methods

Table 2: Instruments Used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Thermo – Orion Star A111
4	Weighing machine	SCALETEC-Model SAB-203L
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 3: Chemicals Used

S.No	Chemical	Company Name
1	Rimantadine	Glen mark
2	KH_2PO_4	FINER chemical LTD
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	MOLYCHEM
5	Ortho phosphoric Acid	MERCK

Mobile Phase Optimization:
Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to pH 3.0 Phosphate buffer and methanol in proportion gradient programme.

Optimization of Column:
The method was performed with various columns like C18 column Phenomenex column, YMC, Inertsil ODS column. XBridge BEH C18 (4.6*250mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized chromatographic conditions:
Equipment : High performance liquid chromatography equipped with Auto Sampler and PDA detector
Column : X Bridge BEH C18 (4.6*250mm, 5µ)
Buffer : pH 3.0 Phosphate buffer
Mobile phase: pH 3.0 Phosphate buffer 30ml: Methanol 70ml
Flow rate : 1.0 ml per min
Wavelength : 265 nm
Injection volume : 20 µl
Run time : 15 min.

Preparation of buffer and mobile phase:
Preparation of buffer:
Preparation of pH 3.0 Phosphate buffer:
6.8 g of KH2PO4 is taken into 1000 ml of HPLC water and adjust to pH 3.0 with NaoH solution ,then filtered through 0.45 µm Membrane filter and sonicate it for 5 mins.

Diluent Preparation: The Mobile phase was used as the diluent.

Preparation of standard solution:
Standard Solution Preparation:
Accurately weigh and transfer 25 mg of Rimantadine working standard into a 25 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm)

Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the area for Rimantadine peak and calculate the %Assay by using the formulae.

System suitability:
Tailing factor for the peak due to Rimantadine in Standard solution should not be more than 2.0
Theoretical plates for the Rimantadine peak in Standard solution should not be less than 2000.
Resolution for the Rimantadine peak in standard solution should not be less than 2.

Calculation: (For Rimantadine)

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} * \frac{\text{WS}}{\text{DS}} * \frac{\text{DT}}{\text{WT}} * \frac{\text{Average weight}}{\text{Label Claim}} * \frac{\text{P}}{100} * 100$$

Where:
AT = average area counts of sample preparation.
AS= average area counts of standard preparation.
WS= Weight of working standard taken in mg.
P= Percentage purity of working standard
LC= Label Claim mg/ml.

Table 4: Sample and Standard Details

S.No	Samples
1	Rimantadine
2	Rimantadine working standard

Method validation summary:

Precision:

Preparation of stock solution: Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm)

Procedure: The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm).

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm)

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 10 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm)

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm).

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 30 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm)

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Rimantadine and calculate the individual recovery and mean recovery values.

Linearity:

Preparation of stock solution: Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (15ppm Rimantadine):

0.15 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (30ppm Rimantadine):

0.3 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (45ppm Rimantadine):

0.45ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (60ppm Rimantadine):

0.6 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (75ppm Rimantadine):

0.75 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Limit of detection (LOD):

Preparation of 0.07µg/ml solution:

Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 1.45 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of quantification:

Preparation of 1.84 µg/ml solution:

Accurately weigh and transfer 20 mg of Rimantadine working standard into a 20 ml clean dry volumetric flask add about 10 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.45ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 5.2 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Procedure for LOD and LOQ:

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.8 ml/min to 1.2ml/min. Standard solution 45 ppm of Rimantadine was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from 63% to 77%: Standard solution 45 ppm of Rimantadine was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10

Table 5: Method Development and QBD Approach

Std	Run	Block	Factor 1 A:flow rate ml/min	Factor 2 B:Buffer PH	Factor 3 C:Organic ratio mi	Response 1 RT min	Response2 Tailing factor
4	1	Block 1	1.00	3.50	65.00	6	1
17	2	Block 1	0.90	3.00	65.00	6.13	1.03
10	3	Block 1	0.90	3.50	60.00	6.14	1.04
8	4	Block 1	1.00	3.00	70.00	6.12	1.02
6	5	Block 1	1.00	3.00	60.00	6.11	1.01
11	6	Block 1	0.90	2.50	70.00	6.09	0.9
2	7	Block 1	1.00	2.50	65.00	6.09	0.9
13	8	Block 1	0.90	3.00	65.00	6.13	1.03
7	9	Block 1	0.80	3.00	70.00	6.12	1.02
15	10	Block 1	0.90	3.00	65.00	6.13	1.03
5	11	Block 1	0.80	3.00	60.00	6.15	1.05
12	12	Block 1	0.90	3.50	70.00	6.13	1.03
3	13	Block 1	0.80	3.50	65.00	6.14	1.04
14	14	Block 1	0.90	3.00	65.00	6.13	1.03
16	15	Block 1	0.90	3.00	60	6.09	0.9
9	16	Block 1	0.90	2.50	65.00	6.09	0.9

Table 6: FIT Summarry

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean	Std. Dev.
A	Flow rate	ml/min	Numeric	0.80	1.00	-1.000	1.000	0.900	0.069
B	Buffer PH		Numeric	2.50	3.50	-1.000	1.000	3.000	0.343
C	Organic Ratio	ml	Numeric	60.00	70.00	-1.000	1.000	65.000	3.430

Table 7: Response 1: Retension Time of Tailing Factor

Response	Name	Units	OBS	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
Y1	RT	min	17	Polynomial	6.00	6.15	6.11	0.034	1.03	None	Quadratic
Y2	Tailing Factor		17	Polynomial	0.90	1.05	1.00	0.055	1.17	None	Quadratic

3. Results and Discussion

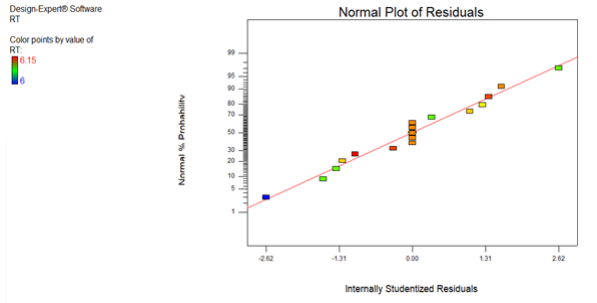

Fig.2: Normal plot of Residuals for Rimantidine

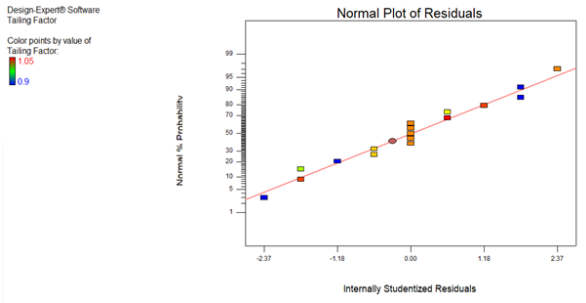

Fig.7: Normal plot of Residuals for Rimantidine

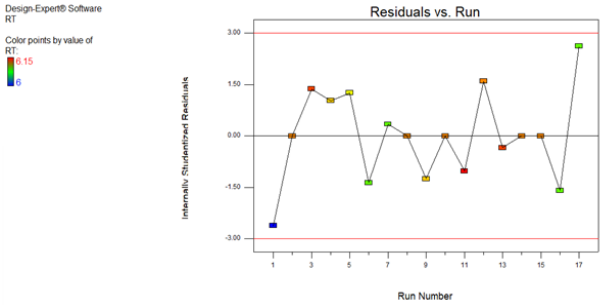

Fig.3: Residual vs. Run for Rimantidine

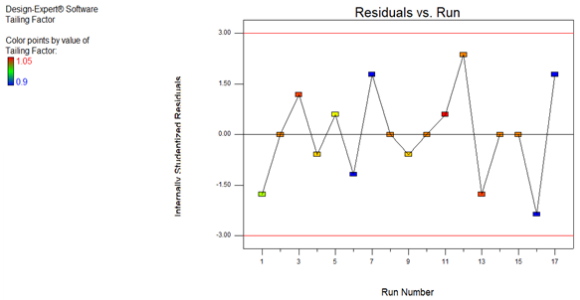

Fig.8: Residual vs. Run for Rimantidine

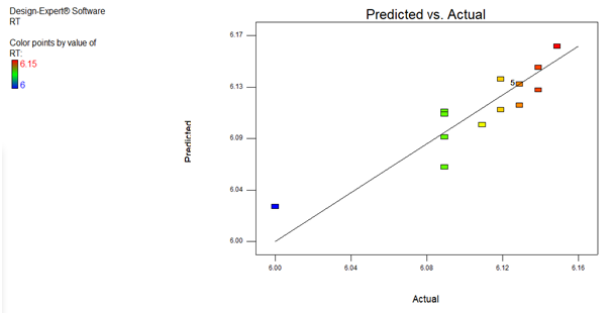

Fig.4: Predicted vs. Actual for Rimantidine

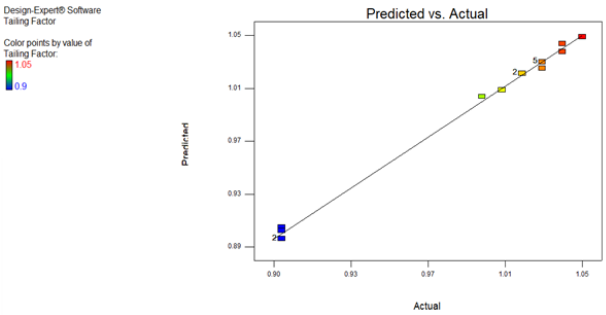

Fig.9: Predicted vs. Actual for Rimantidine

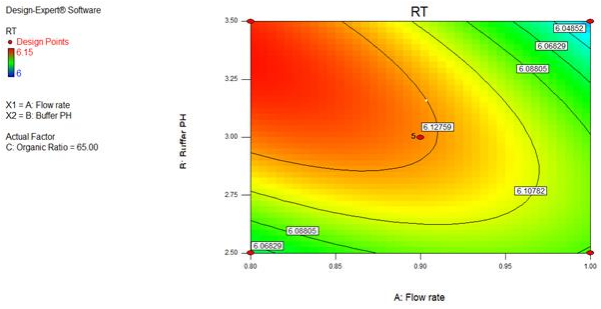

Fig.5: Retention time for Rimantidine

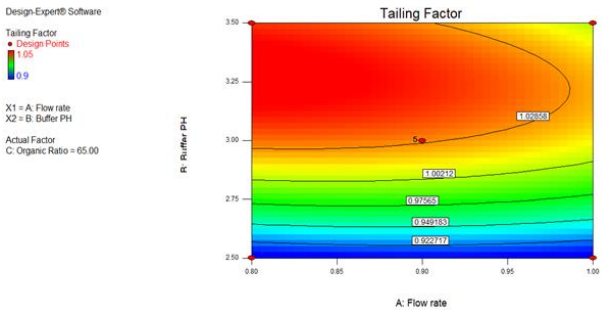

Fig.10: Retention time for Rimantidine

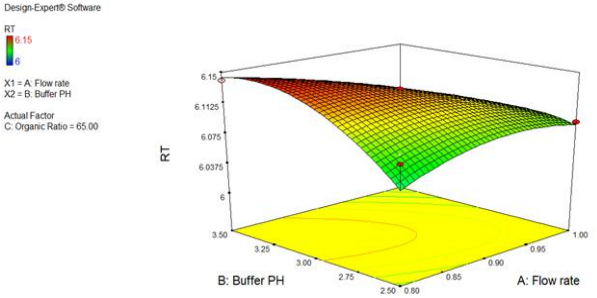

Fig.6: 3D Surface for Rimantidine

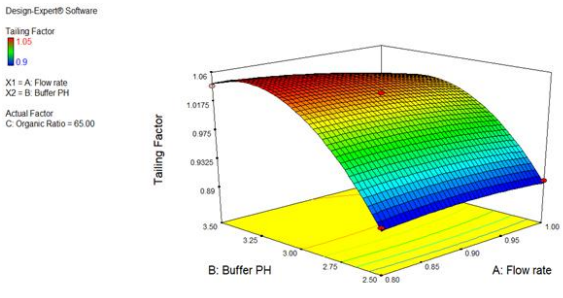

Fig.11: 3D Surface for rimantidine

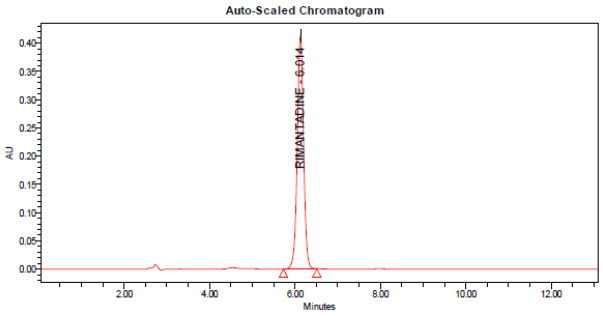

Fig.12: Chromatogram for Sample

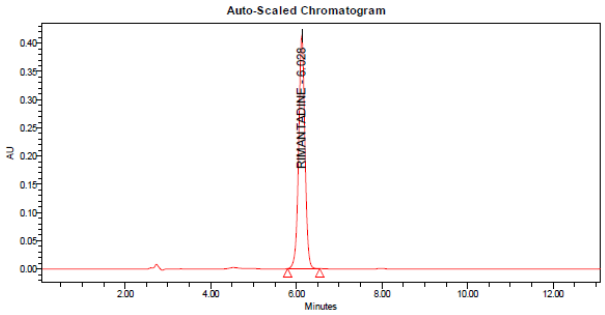

Fig.13: Chromatogram for Standard

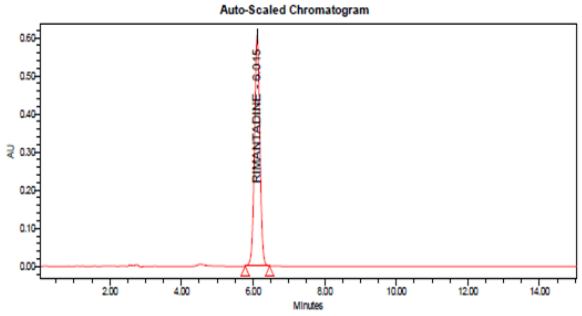

Fig.14: Chromatogram of L-5

Table 8: Linearity results

S. No	Linearity Level	Concentration (µg/ml)	Area
1	I	15	4698102
2	II	30	9196240
3	III	45	14694358
4	IV	60	19392458
5	V	75	23993500
Correlation Coefficient			0.9994

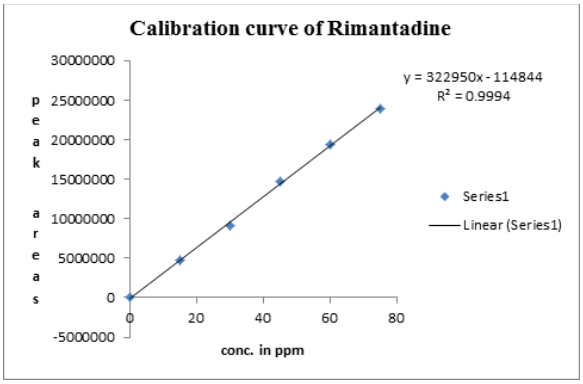

Fig.15: Calibration graph of Rimantadine

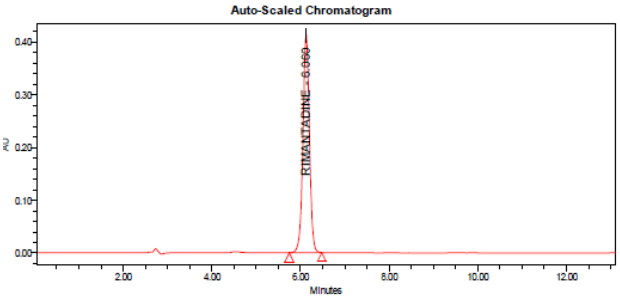

Fig.16: Chromatogram for Precision -6

Table 12: The results are summarized for Rimantadine

Injection	Areas
Injection-1	14735896
Injection-2	14758742
Injection-3	14798562
Injection-4	14735478
Injection-5	14724587
Injection-6	14798741
Average	14758667.67
Standard Deviation	32909.68455
%RSD	0.2

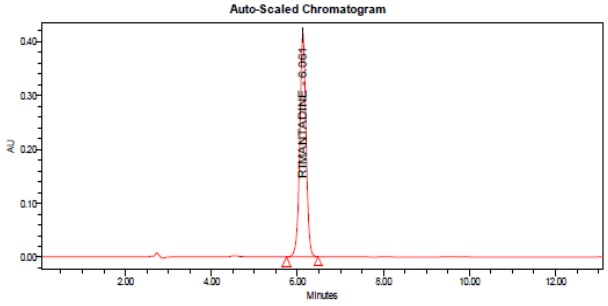

Fig.17: chromatogram for ID precision -6

Table 9: ID Precision Result

Injection	Areas
Injection-1	14845217
Injection-2	14821458
Injection-3	14865848
Injection-4	14854217
Injection-5	14836985
Injection-6	14854128
Average	14846308.83
Standard Deviation	15563.72312
%RSD	0.1

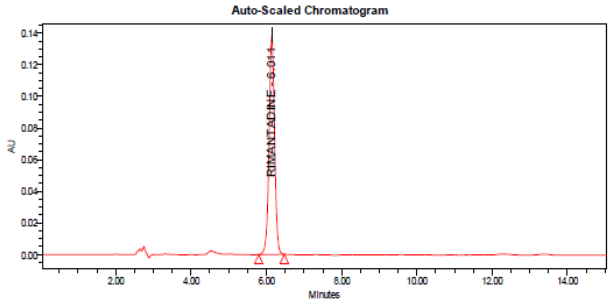

Fig.18: Chromatogram for Accuracy 50%

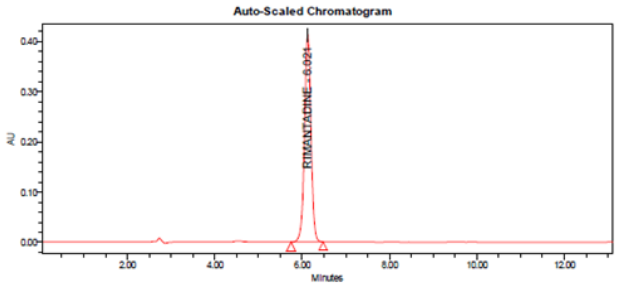

Fig.19: Chromatogram for Accuracy 100%

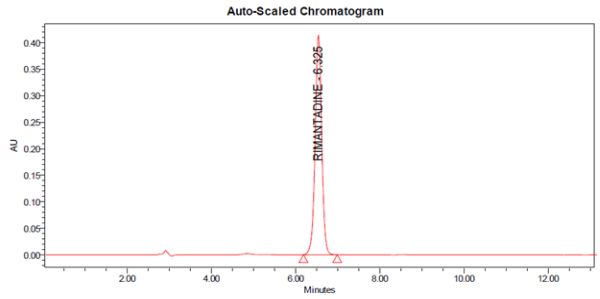

Fig.23: Chromatogram Showing Less Flow

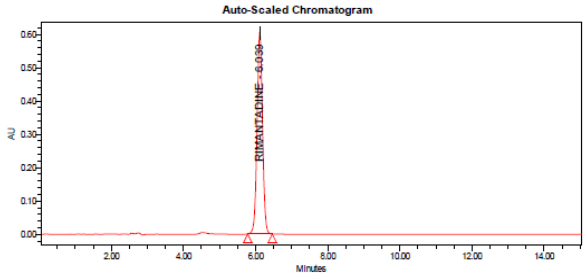

Fig.20: Chromatogram for Accuracy 150%-

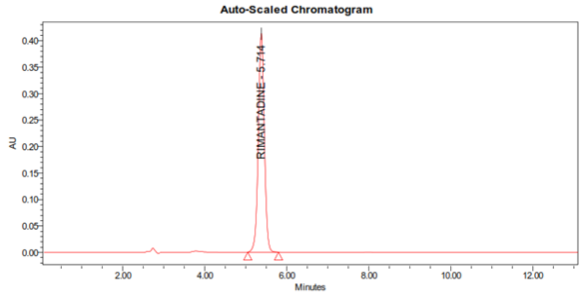

Fig.24: Chromatogram Showing More Flow

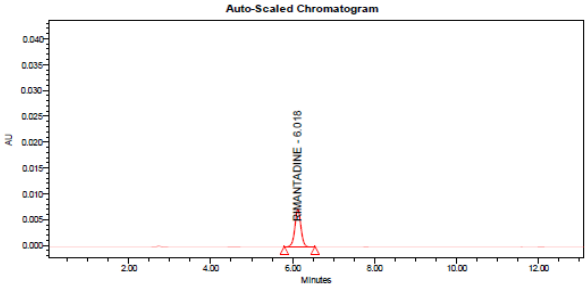

Fig.21: Chromatogram of Rimantadine Showing LOD

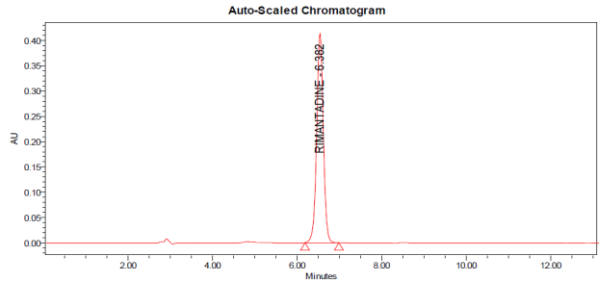

Fig.25: Chromatogram Showing Less Organic

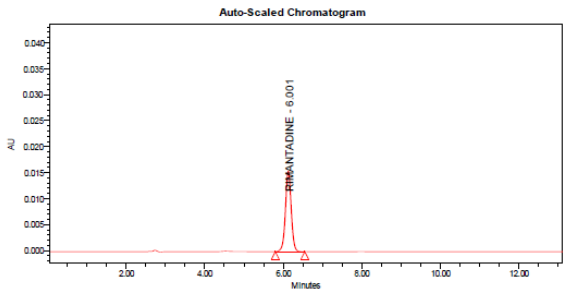

Fig.22: Chromatogram of Rimantadine Showing LOQ

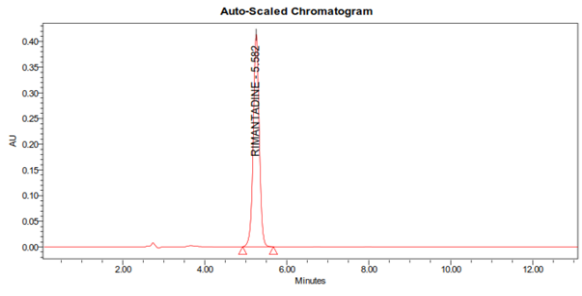

Fig.26: Chromatogram Showing More Organic

Table 10: The Accuracy Results

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	7511587	10	10.12	101.2	98.6
100%	14021697	20	19.6	97.8	
150%	21533875	30	29.0	96.7	

Table 11: Robustness studies: Flow change

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5812	1.2
2	1.0	5843	1.02
3	1.2	5891	1.5

Table 12: Robustness studies: Organic composition change

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5812	1.2
2	*Actual	5843	1.02
3	10% more	5891	1.5

4. Conclusion

A simple, precise, and robust RP-HPLC method was successfully developed and validated for the estimation of Rimantadine in bulk and pharmaceutical dosage forms, in line with ICH Q2(R1) guidelines. Method optimization revealed that flow rate, buffer pH, and organic ratio significantly influenced retention time and tailing factor. The optimized chromatographic conditions employed an Inspire C18 column (3.0 × 150 mm, 5 µm) with a mobile phase of phosphate buffer (pH 3.0) and methanol at a flow rate of 1.0 mL/min, detection at 265 nm, and an injection volume of 10 µL. A sharp, symmetrical peak was obtained at 6.12 min with satisfactory system suitability parameters (plate count >5800, tailing factor ~1.0). Validation studies confirmed the accuracy, precision, and sensitivity of the method. Linearity was excellent over the range of 15–75 µg/mL with R^2 = 0.9994. Precision and intermediate precision showed %RSD<0.2%, confirming reproducibility. Accuracy studies at 50%, 100%, 150% levels demonstrated recoveries between 96.7–101.2%, within the acceptance criteria. The LOD (2.96µg/mL) and LOQ (9.97 µg/mL) confirmed good sensitivity, while robustness studies indicated that small changes in flow rate and organic composition did not significantly affect chromatographic performance. Overall, the developed method is simple, reliable, stability-indicating, and suitable for routine quality control, batch release, and regulatory analysis of Rimantadine formulations.

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