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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE ANALYSIS OF RIMANTADINE HYDROCHLORIDE IN MEDICINAL FORM

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ABSTRACT

The aim of the research work was to conduct precolumn derivatization of Rimantadine hydrochloride (RMT) with Anthraquinone – 2 – sulphonyl chloride (AQSC) to develop a validated, selective, precise and accurate RP-HPLC – UV method for the analysis of RMT – AQSC derivative in its Medicinal form.

The isocratic mobile phase for the C – 18 column consisted of Ethylnitrile and 0.005M 1 – octane sulfonic acid sodium salt monohydrate buffer (pH adjusted to 6.7) in 60:40 volume ratio. Flow rate maintained at one millilitre in one minute at ambient temperature. The ideal UV detection wavelength for RMT derivative was 259 nm.

The retention time for both API and the medicine was 6.79 minutes. Linearity was satisfied over a range of 50 ppm to 250 ppm with a correlation coefficient (r) value 0.999. Percentage RSD for precision, accuracy and robustness were less than 2. The LOD and LOQ were 1.32 ppm and 4.0 ppm respectively. Validation was done as per 1CH guidelines and all the results were with in the limits.

Keywords: RP-HPLC, Method validation, Derivatization (DRT), ICH.

INTRODUCTION

RMT (Fig.-1) $C_{12}H_{22}ClN$, molecular weight, 215.76 g/mol is 1-(1-Adamantyl) Ethanamine Hydrochloride .It is an antiviral drug against influenza virus A and prophylaxis in children and is taken orally. By resisting the breakage of the protective shells of the virus, RMT stops viral multiplication in the host cell. RMT alone shows no absorption in the UV – visible region, hence it is derivatized[1] to enable HPLC detection. The earlier works for the determination of RMT included analysis of RMT by GC – MS[2], and using capillary zone electrophoresis[3].

New separation method for tricyclic antiviral drugs was developed[4]. Previous works included determination of RMT using IR Analysis[5], online post column DRT[6], fluorescent probe[7], UV-visible spectrophotometry[8], UHPLC coupled with orbitrap mass spectrometry[9], use of AQSC for RMT analysis[10], and simultaneous analysis using tandem mass spectrometry[11]. The present work focused on RP-HPLC method development[12] and its validation[13].

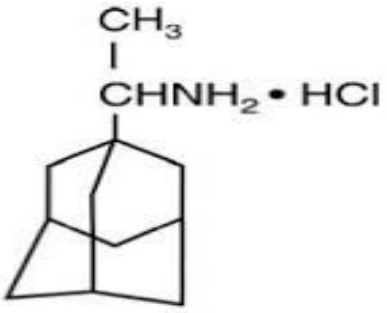

Fig.-1:Rimantadine hydrochloride (RMT)

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EXPERIMENTAL

Chemicals

A sample of RMT (API) was received from a Biopharm Limited in West India. Flumadine® tablets were bought from the pharmacy. AQSC was prepared in the laboratory. Octane sulfonic acid (OSA), sodium hydroxide (SH), dichloromethane (DCM), Hydrochloric acid (HCl), Anhydrous sodium sulfate (SS) orthophosphoric acid, Ethylnitrile (EN) all suitable for HPLC analysis were purchased from Sigma Aldrich limited.

Table-1: Instrumentation

Column	C – 18 (250 mm × 4.6 mm)
Decteror	UV – VIS, Japan
Pump	LC – 10 ATVP, Japan
Injection valve	7725i model, 20 units
Syringe	50 units.
Software	Baseline N2000

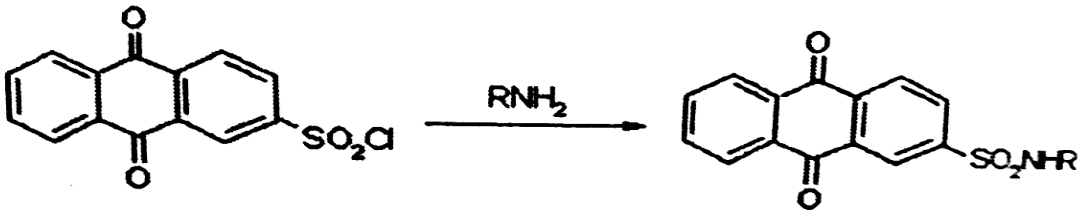
Table-2: Chromatographic Conditions

Mobile Phase	EN : 0.005M OSA 60:40(V/V)
Flow rate (FR)	1 ml per minute
Injection volume	20 units
Wavelength	260 nm
Elution type	Isocratic
Buffer pH	6.7
Temperature	Ambient

Preparation of Reagents

DRT of RMT (API and sample)

RMT 50mg was dissolved in water, mixed with SH in a 100ml flask and stirred. DCM 15ml and AQSC (1.0 millimolar in 25ml DCM) were added to the above flask within 30 minutes and stirred for one hour. The aqueous phase was removed, the organic phase was washed three times with 1M HCl and dried over SS. DCM was removed by rotatory evaporation to give a residue which was RMT – AQSC derivative and it was subjected to RP – HPLC analysis(DRT Reaction, Fig.-2).



AQSC Derivative

Fig.-2

Preparation of stock and working standard (WS) solutions

The diluent used for all solutions was EN. RMT – AQSC derivative prepared from RMT (AP1) 50mg was diluted with EN up to the mark in a 50ml volumetric flask. The concentration of this solution was 1000 microgram per milliliter or 1000 ppm (part per million). From the stock solution, WS solution of 150 ppm was prepared by suitable dilution in a 10 ml flask. All the prepared solution were micro-filtered and sonicated for sufficient time.

Preparation of Sample Solution

Ten Flumadine® tablets containing RMT active ingredient were weighed and their average weight was noted. All the tablets were powdered and a part of the powder equal to 50mg of RMT as given in the label information of the tablets was separated and subjected to DRT with AQSC to form the derivative, which

was transferred into a 50ml flask and diluted with EN up to the mark to result in a 1000 ppm solution. 150 ppm sample solution was prepared from the above solution by suitable dilution.

Mobile Phase preparation

0.005M OSA ($C_8H_{17}NaO_3S.H_2O$, 234.3 gm/mol) buffer solution was prepared in HPLC grade water with pH adjusted to 6.7. Mobile phase composed of 60:40 v/v ratio of EN and OSA buffer.

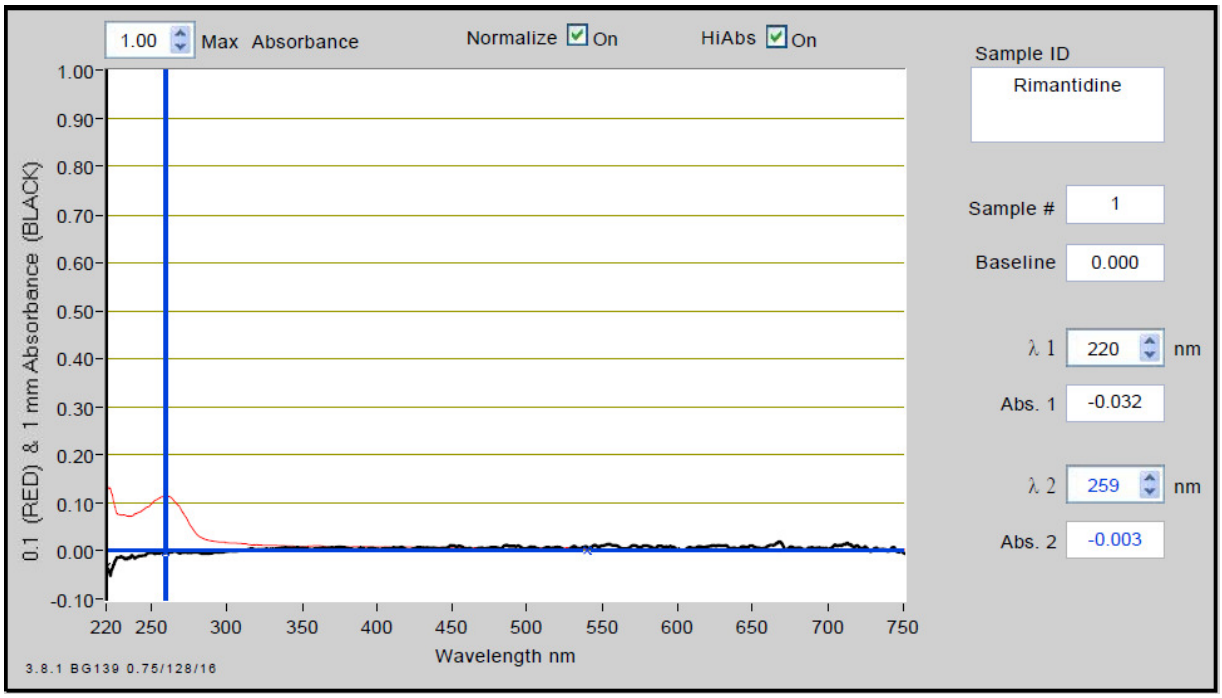


Fig.-3: UV-Spectrum of RMT - Derivative

System Suitability (ST)

To know the resolution and reproducibility of the method ST Study was done.

RMT (WS) was injected into the HPLC system to facilitate the study of 6 different chromatograms. The acceptance criteria for resolution (Rs), Tailing factor (T), Asymmetry (K), Theoretical plates (N), standard deviation (SD) and % RSD percent relative standard deviation for Retention time (RT), Peak area (PA) and Peak height (PH) were checked (Table-3).

Table-3: System Suitability

ST Parameter	Reference Value	Results
RT	% RSD ≤ 1	0.0601
PA (n = 6)	% RSD ≤ 1	0.9745
PH	% RSD ≤ 1	0.51721
Rs	Rs ≥ 2.0	12.956
T	T ≤ 2.0	1.048
K	K ≤ 2.0	1.075
N	N ≥ 2000	7720.263

Estimation of RMT in sample

The Response factor (RF) of the WS and the sample were calculated separately from the average of six peaks to calculate the amount of the drug.

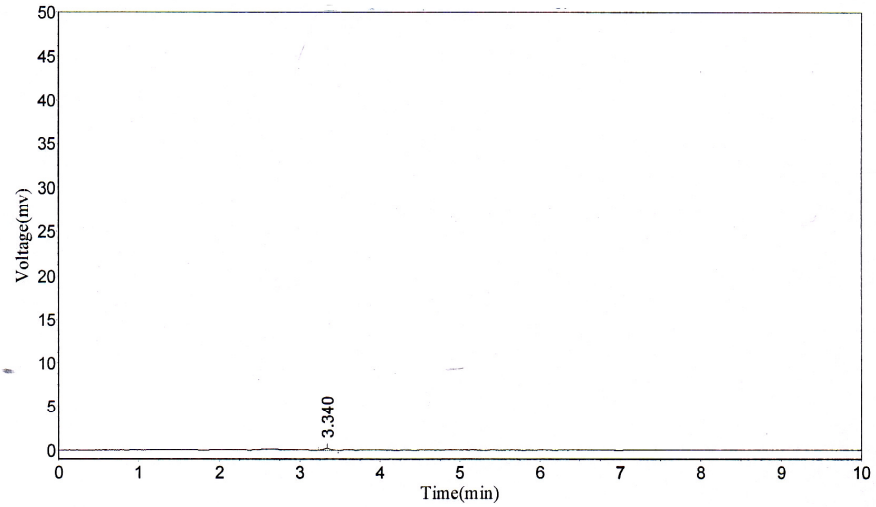
$$\text{Amount of the drug} = \frac{\text{R.F of the sample}}{\text{R.F of the WS}} \times \text{strength of the W.S}$$

Method Validation

Validation was done as per 1CH Q2(R1) guidelines for linearity, Accuracy, Precision, Robustness, Limit of detection (LOD) and limit of Quantification (LOQ).

RESULTS AND DISCUSSION

All the ST measurements met the criteria (as per given in Table-3). RT of the Blank (Mobile phase) was 3.34 minutes (Fig.-4). RT of the RMT (WS) was 6.79 minutes (Fig.-5). RT of the RMT (sample) was also 6.79 minutes (Fig.-6).



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		3.340	211.600	1091.000	100.0000
Total			211.600	1091.000	100.0000

System Evaluation

Peak No.	Peak ID	Ret. Time	Half-Peak Width	Theoretical levels	Resolution	Tail Factor	Asymmetry
1		3.340	0.078	10071.855	0.000	1.143	1.341

Fig.-4: Blank Chromatogram

Linearity

Five solution levels ranging from 50 ppm to 250 ppm were prepared from the WS. Each level in the range was injected thrice. The calibration curve of PA versus strength in ppm was plotted. The linear regression equation and the correlation coefficient (r) value depicted the linear relationship between PA and the concentration of the solution. (Table-4 and Fig.-7).

Accuracy

80%, 100% and 120% of WS solutions were spiked to sample solution and each injected thrice. The average PA study depicted a percent recovery of 101.68, 101.87 and 98.45 from each level of addition and was within the acceptance limits (Table-5).

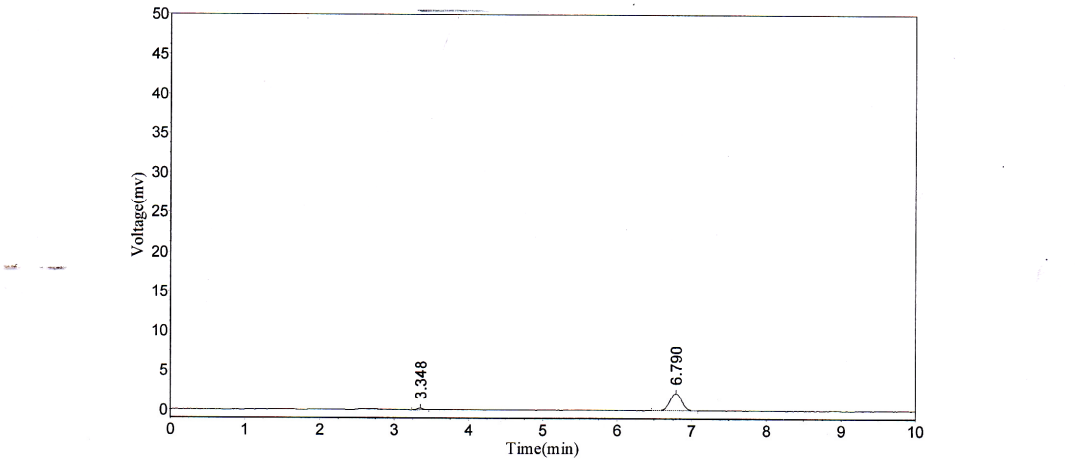
Table-4: Linearity

Strength(ppm)	PA(n = 3)	% RSD
50	24762.93	0.4401
100	50324.16	0.504695
150	72088.5	0.4077
200	97907.76	0.861
250	121061.60	0.750228

Regression line Equation Y = 483.7x + 559.3	R^2 = 0.999
Slope (m) 483.7	Intercept (c) = 559.3

Table-5: Accuracy

Sample	% of WS	Amount WS (ppm)		PA (n = 3)	% RSD	Recovery (%)
		Spike	Found			
1	80	120	122.019	58916.9	1.00	101.68
2	100	150	152.818	73788.6	0.292	101.87
3	120	180	177.225	85573.5	0.639	98.45



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		3.348	223.000	1141.900	4.4295
2		6.790	2187.840	24637.801	95.5705
Total			2410.840	25779.701	100.0000

System Evaluation

Peak No.	Peak ID	Ret. Time	Half-Peak Width	Theoretical levels	Resolution	Tail Factor	Asymmetry
1		3.348	0.080	9704.813	0.000	1.059	1.091
2		6.790	0.180	7883.231	13.237	1.043	1.099

Fig.-5: RT of the RMT (WS)

Precision

All precision studies were done with 150 ppm sample solution.

Six separate chromatograms took on the same day (Intraday precision) Table-6 and an average of three chromatograms taken separately on three different days (Interday precision) Table-7 were studied to know the % RSD of RT and PA. All the values met the criteria and confirm the closeness of the data to each other and the method was precise.

Table-7 were studied to know the % RSD of RT and PA. All the values met the criteria and confirm the closeness of the data to each other and show that the method was precise.

LOD

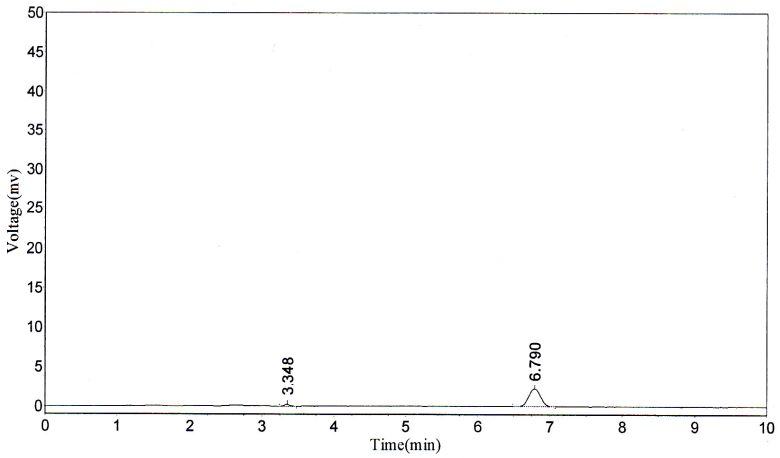
The lowest concentration of the sample that can be detected under the developed conditions was calculated from the regression line as 1.32 ppm.

LOQ

The lowest concentration of the sample that can be detected with adequate precision and accuracy was 4.0 ppm. This was also calculated from the regression line.

Robustness

A small variation in the experimental conditions (Table-8) showed little or no effect on the average PA, RT, and % RSD values and hence the method was Robust (Table-9).



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		3.348	204.379	1049.300	4.0535
2		6.790	2204.400	24837.199	95.9465
Total			2408.779	25886.499	100.0000

System Evaluation

Peak No.	Peak ID	Ret. Time	Half-Peak Width	Theoretical levels	Resolution	Tail Factor	Asymmetry
1		3.348	0.080	9704.813	0.000	1.174	1.275
2		6.790	0.178	8031.270	13.323	1.048	1.099

Fig.-6: Sample Chromatogram

Table-6: Intraday Precision

Trial	PA	RT	PH
1	74380.2	6.78	6533.1
2	74605.7	6.78	6543.0
3	72692.1	6.78	6453.7
4	74263.9	6.78	6527.1
5	74182.2	6.78	6519.9
6	74611.9	6.79	6543.11
Mean	74122.7	6.7816	6520.02
SD	722.37	0.0040	33.722
% RSD	0.974	0.060	0.5172

Table-7: Interday Precision

Day	1	2	3
Trial	PA	PA	PA
1	74128.3	74679.1	78485.7
2	73211.8	75164.7	77627.5
3	73323.7	75094.2	77530.7
Mean	73554.6	74979.3	77881.33
SD	500.01	262.42	525.62
% RSD	0.6797	0.3499	0.6749

Table-8: Experimental Conditions

Value	I	II	III
Buffer pH	6.6	6.7	6.8
Column Temp (ºC)	34	35	36
Flow rate (ml/min)	0.98	1.00	1.02

Table-9: Robustness

Value	pH			Temp			Flow rate.		
	6.6	6.7	6.8	34	35	36	0.98	1.00	1.02
Mean PA (n = 3)	76042.0	75090	76051	76001	75561	77490	79169	77251	76229
SD	279.60	506.2	176.08	617.0	477.7	296.7	136.2	686.9	191.2
% RSD	0.367	0.6742	0.2315	0.811	0.6322	0.38	0.172	0.889	0.250

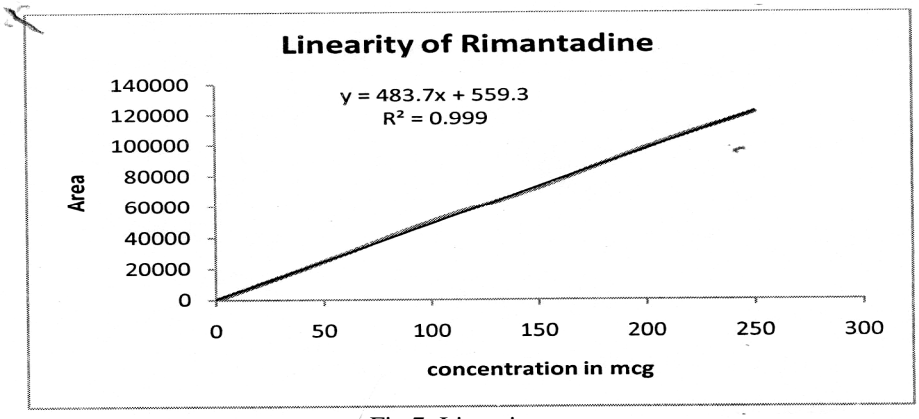


Fig.7: Linearity

CONCLUSION

The developed RP-HPLC method was selective, accurate, precise, and robust. All the validation measurables for the API and the sample were similar. The method can be used in quality control test.

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