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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF TOLVAPTAN IN BULK AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-LC) method has been developed and subsequently validated for the determination of Tolvaptan in Bulk and its pharmaceutical formulation. Separation was achieved with an AMCHEMTEQ-USA-ACI C18 (150 mmx4.6 mm I.D; particle size 5 µm)Column and Water: Acetonitrile (40:60) as eluent at flow rate 1.0 mL/min. UV detection was performed at 254nm. The method is simple, rapid, and selective. The described method of Tolvaptan is linear over a range of 37.285 µg/mL to 298.282 µg/mL. The method precision for the determination of assay was below 2.0%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 99.6 to 101.1. The method is useful in the quality control of Bulk and pharmaceutical formulations.

Key Words: Tolvaptan, Estimation, RP-HPLC, Validation, Tablets.

INTRODUCTION

Tolvaptan[1-2] is indicated for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (serum sodium < 125 mEq/L or less marked hyponatremia that is symptomatic and has resisted correction with fluid restriction), including patients with heart failure, cirrhosis, and Syndrome of Inappropriate Antidiuretic Hormone .

Chemically (±)-4'-[(7-chloro-2, 3, 4, 5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-*o* tolu-*m*-toluidide (Fig-1). Tolvaptan is a white to off white crystalline powder with a Molecular weight 448.94. Tolvaptan is soluble in benzyl alcohol and methanol, practically insoluble in water and hexane. Tolvaptan melting point was approximately 224°c. Its empirical formula is $C_{26}H_{25}ClN_2O_3$. It is not official in any pharmacopoeia, few liquid chromatography procedures have been reported for the determination of Tolvaptan[3-4] .The author have developed a liquid chromatographic method which would serve as a rapid and reliable method for the determination of Tolvaptan in Bulk and pharmaceutical dosage forms.

EXPERIMENTAL

The Experimental[5-7] involves the followings-

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column AMCHEMTEQ-USA-ACI C18 (150 mmx4.6 mm I.D; particle size 5 µm) was used.

Chemicals and solvents

The HPLC Grade water (Millipore) and Acetonitrile HPLC Grade from E. Merck (India) Ltd., Mumbai.

Mobile phase preparation

Prepare a filtered and degassed mixture of Water and Acetonitrile in the ratio 400:600 v/v respectively.

Standard preparation: (For Tolvaptan tablets 15mg & 30mg)

Accurately weigh and transfer about 30.0mg of Tolvaptan working standard into a 200 mL volumetric flask, add 120 mL of mobile phase and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with mobile phase.

Sample preparation: (For Tolvaptan tablets 15mg)
Transfer 5 tablets (equivalent to 75 mg of Tolvaptan) into a 250 mL volumetric flask add about 100 mL of mobile phase sonicate for 20minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with mobile phase. Filter the solution through 0.45um Filter. Transfer 5.0 mL of the filtered solution into a 10 mL volumetric flask and dilute to volume with mobile phase.
Sample preparation: (For Tolvaptan tablets 30mg)
Transfer 5 tablets (equivalent to 150 mg of Tolvaptan) into a 250 mL volumetric flask add about 100 mL of mobile phase, sonicate for 20minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with mobile phase. Filter the solution through 0.45um Filter. Transfer 5.0 mL of the filtered solution into a 20 mL volumetric flask and dilute to volume with mobile phase.
Chromatographic conditions: An AMCHEMTEQ-USA-ACI C18 (150 mmx4.6 mm I.D; particle size 5 µm) column was used for analysis at column temperature 25°C. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 254nm for the detection and Chromatographic runtime was 10minutes.

RESULTS AND DISCUSSION

Method development[5-7]
To develop a suitable and robust LC method for the determination of Tolvaptan in different columns and flow rates were employed to achieve the best separation and resolution. The method development was started with AMCHEMTEQ -USA-ACI C18 (250 mmx4.6 mm I.D; particle size 5 µm) column with flow rate of 1.5ml/minute. The retention time of Tolvaptan was 5.224minutes. For further reducing the runtime short column was used with same flow rate AMCHEMTEQ -USA-ACI C18 (150 mmx4.6 mm I.D; particle size 5 µm). The chromatogram of Tolvaptan standard using the proposed method is shown in Fig-2. System suitability results of the method are presented in Table-1. Tolvaptan show significant UV absorbance at Wavelength 254nm. Hence this wavelength has been chosen for detection in analysis of Tolvaptan.
Column selection
Based on the retention and better peak shape of the compound AMCHEMTEQ-USA-ACI C18 (150 mmx4.6 mm I.D; particle size 5 µm) column was selected as suitable column for analysis of Tolvaptan.
Method validation[6-7]
The developed LC method extensively validated for assay of Tolvaptan using the following parameters.
Specificity
Blank and Placebo interference: A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of Blank and Placebo solutions showed no peaks at the retention time of Tolvaptan peak. This indicates that the excipients used in the formulation do not interfere in estimation of Tolvaptan in Tolvaptan tablets. The chromatogram of Tolvaptan Blank and Placebo using the proposed method is shown in Fig- 3 & Fig-4.
Linearity of detector response
Linearity of detector response was established by plotting a graph to concentration *versus* average area and determining the correlation coefficient. A series of solutions of Tolvaptan standard were prepared in the concentration range of about 37.285 µg/mL 298.282 µg/ mL. A graph was plotted to concentration in µg/mL on X-axis *versus* response/Area on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.9999. Linearity graph is shown in Fig-5. Linearity results of the method are presented in Table-2.
Precision of test Method
The precision of test method was conducted by assay in six samples of Tolvaptan dispersible tablets. The average % assay of Tolvaptan in Tolvaptan tablets was found to be 100.0, 100.2, for 15 & 30 mg tablets respectively and the %RSD is 0.8 and 0.6%. The results were given in Table-3. A typical LC Chromatogram is shown in Fig-6.
Accuracy

A Study of recovery of Tolvaptan from spiked placebo was conducted at six different spike levels i.e.25, 50, 75,100, 125 and 150%. Samples were prepared by mixing placebo with Tolvaptan raw material equivalent to about the target initial concentration of Tolvaptan. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table-4. The mean recoveries of Tolvaptan from spiked were found to be in the range of 99.6-101.1%.

Ruggedness

A study to establish the stability of Tolvaptan in standard and test solutions were conducted on bench top and refrigerator at Initial, 1 day and 2 day. The assay of Tolvaptan in standard and test solutions were estimated against freshly prepared standard each time. The difference in% assay of standard and test solutions from initial to 1 day and 2 days was calculated and given in Table-5(Bench Top) and Table-6(Refrigerator). From the above study, it was established that the Standard and sample preparations are stable for a period of 48hours at room temperature (25°C±2°C) and at refrigerator condition (2°C-8°C).

Robustness

A study to establish the effect of variation in mobile phase composition, flow, and Temperature was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated. From the above study the proposed method was found to be robust.

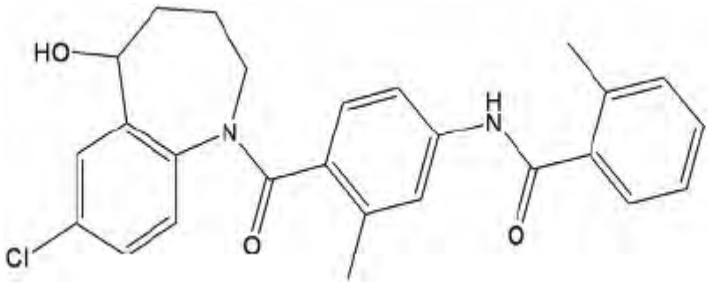

Fig-1: Chemical Structure of Tolvaptan

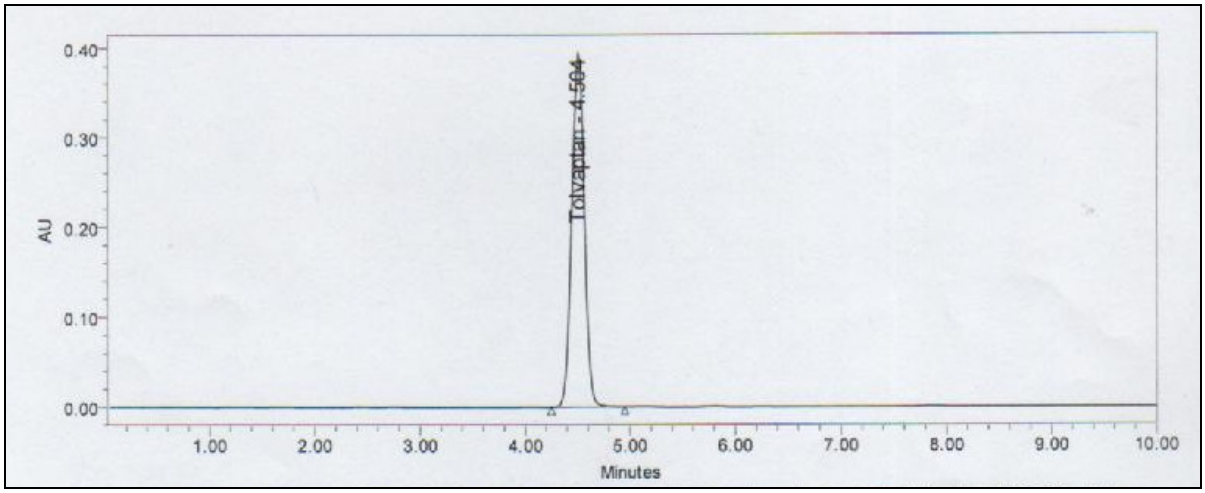

Fig-2: HPLC Chromatogram of Tolvaptan Standard.

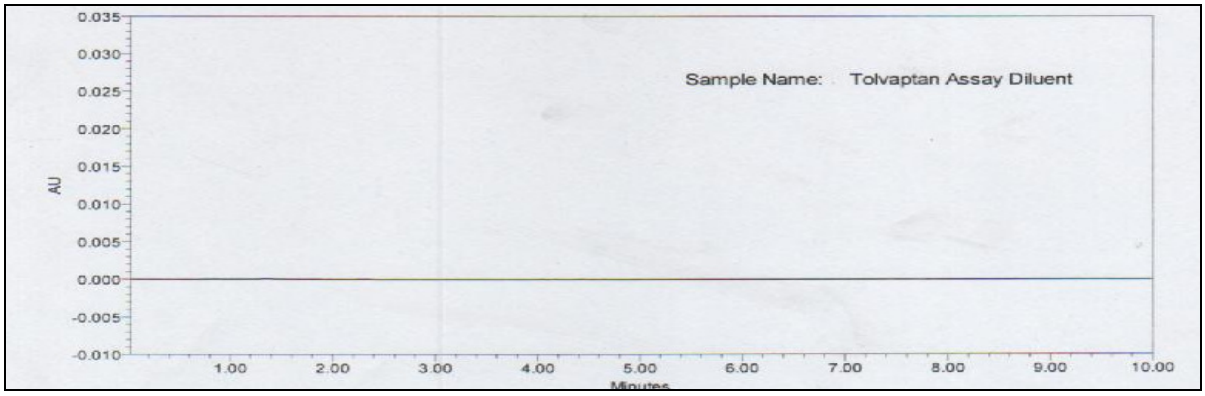

Fig-3: HPLC Chromatogram of Tolvaptan Blank

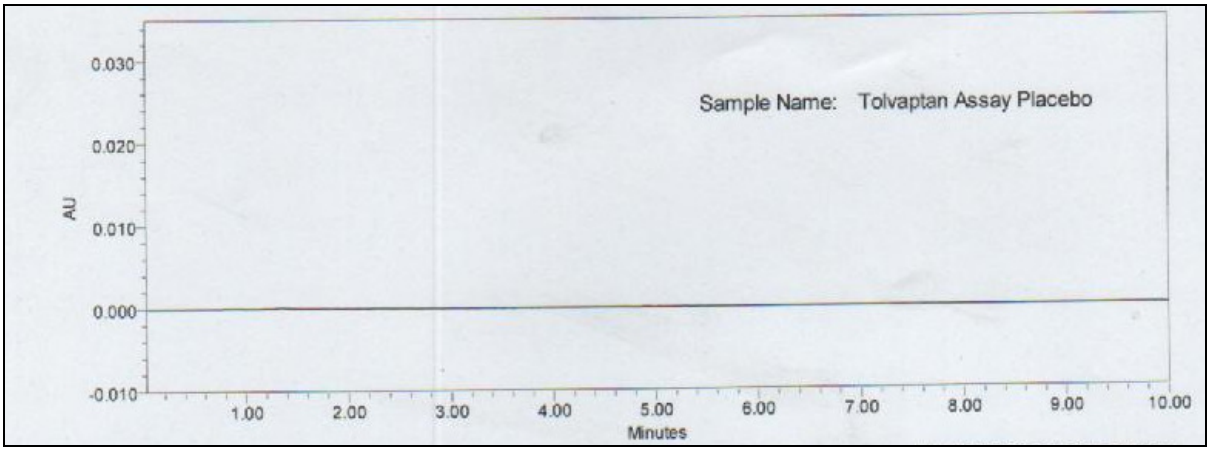

Fig-4: HPLC Chromatogram of Tolvaptan placebo.

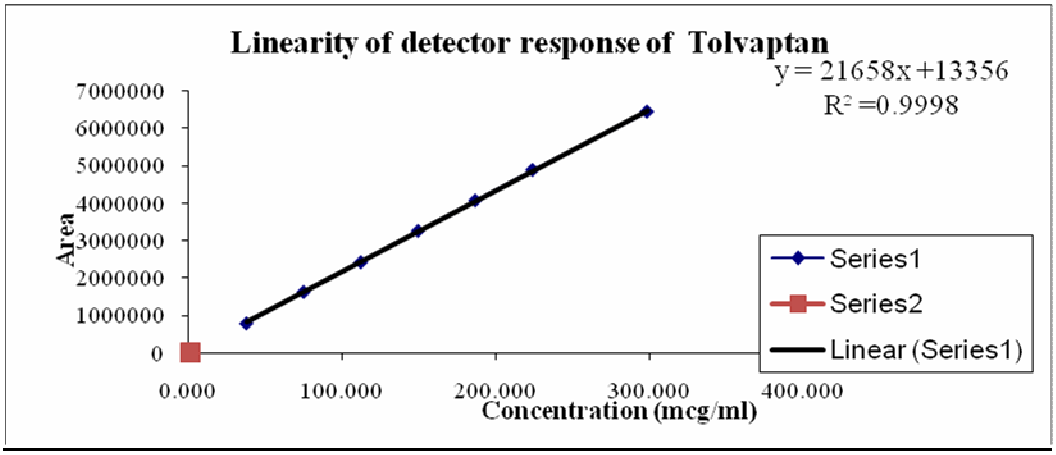

Fig-5: Linearity of detector response graph.

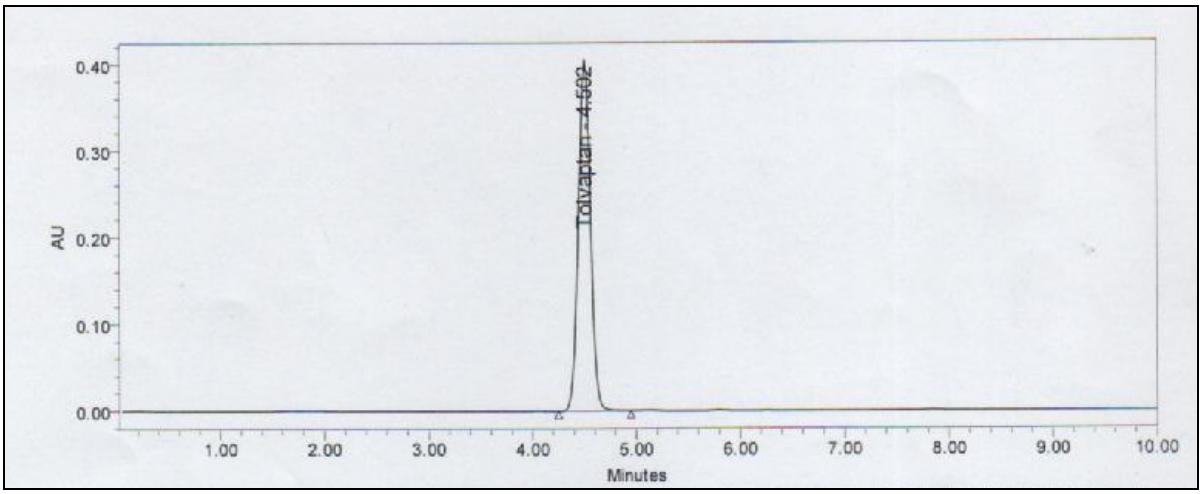

Fig-6: Typical LC chromatogram of Formulated Tolvaptan tablets 30mg.

Table-1: System suitability report

Compound	Retention Time * (min.)	Tolvaptan area/response*	USP Tailing*	USP Plate count*	%RSD*
Tolvaptan	4.505	3242491	1.01	6929	0.70

*Number of standard injections analysed are six.

Table-2: Linearity Table Report

% Level	Concentration(mcg/ml)	AREA	y-Best fit	$(Difference)^2$	Correlation Coefficient (R)=	0.9999
25	37.285	795865	820884	625950401	Regression Coefficient (R^2)=	0.9998
50	74.571	1633992	1628434	30892592	y-Intercept=	13356
75	111.856	2426409	2435962	91262101	Slope of Regression line=	21658
100	149.141	3260276	3243490	281758031	Residual Sum of squares=	3733013271.0
125	186.427	4073278	4051040	494518008	Minimum =	37.285
150	223.712	4886416	4858568	775484949	Maximum =	298.282
200	298.282	6435768	6473625	1433147188	y-Intercept at 100%level	0.4

Table-3: Results for precision of test method

Sample No	%Assay (15.0mg)	%Assay (30.0mg)
01	100.1	99.9
02	99.3	101.1
03	99.4	99.8
04	101.5	100.4
05	99.7	99.5
06	100.2	100.6
Average	100.0	100.2

SD	0.8042	0.5913
% RSD	0.8	0.6

Table-4: Accuracy in the assay determination of Tolvaptan

Sample No.	Spike level	'mcg/mL' added	'mcg/mL' found (recovered)	% of Recovery	Mean % recovery
1.	25%	35.8354	36.4123	101.6	101.1
2.	25%	35.8196	36.4098	101.6	
3.	25%	35.8544	35.9250	100.2	
4.	50%	74.2749	74.7582	100.7	99.9
5.	50%	74.2759	74.1683	99.9	
6.	50%	74.2769	73.6868	99.2	
7.	75%	111.2625	111.0127	99.8	99.6
8.	75%	111.3614	110.2889	99.0	
9.	75%	111.5592	111.4772	99.9	
10.	100%	148.3500	149.1637	100.5	100.5
11.	100%	148.4489	148.7906	100.2	
12.	100%	148.5478	149.6594	100.7	
13.	125%	185.4375	186.3601	100.5	100.3
14.	125%	185.1408	186.2818	100.6	
15.	125%	185.0419	184.4787	99.7	
16	150%	222.6239	223.5626	100.4	100.4
17	150%	222.7228	223.5033	100.4	
18	150%	222.8217	223.5310	100.3	

Table- 5: Bench top Stability of Tolvaptan Test preparation and Standard Preparation:

Time	% Assay of Standard preparation	Difference	% Assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Initial	98.9®	NA*	100.0	101.2	NA*	NA*
After 24 hours	99.3	0.4	101.0	102.0	1.0	1.2
After 48 hours	99.9	1.0	101.5	102.5	1.5	1.3

NA*----Not Applicable, ®--------Potency of Tolvaptan on as is basis.

Table- 6: Refrigerator Stability of Tolvaptan Test preparation and Standard Preparation:

Time	% Assay of Standard preparation	Difference	% Assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Initial	98.9®	NA*	100.0	101.2	NA*	NA*
After 24 hours	99.1	0.2	100.8	101.7	0.8	0.5
After 48 hours	99.8	0.9	101.2	101.9	1.2	0.7

NA*----Not Applicable, ®--------Potency of Tolvaptan on as is basis.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Tolvaptan and can be reliably adopted for routine quality control analysis of Tolvaptan in Bulk and its pharmaceutical formulations.

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