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[Research article]

Analytical Method Development and Validation of Tolvaptan in Bulk and Tablet Dosage Form by RP-HPLC

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ABSTRACT

A new, simple, accurate, precise, robust, specific, sensitive and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of TOLVAPTAN in pharmaceutical dosage forms. A Nucleosil C18 with mobile phase containing 0.01M sodium dihydrogen phosphate and acetonitrile in the ratio of 60:40 was used. The flow rate was 0.6 ml / min and wavelength was monitored at 269 nm. Chromatogram showed the main peak at a retention time of 3.055 min. The developed method was validated according to ICH guidelines and validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. The linearity was found to be in the range of 25 to 150 mcg / ml. respectively. Recovery of Tolvaptan was found to be in the range of 99.74-99.87% %.The system precision and method precision was found to be within limits with % RSD of 0.773 and 0.024% .The developed method was found to be cost effective and was successfully employed for the determination of the same in various formulations.

Key Words: Tolvaptan, RP -HPLC, UV detection, Validation.

INTRODUCTION

Tolvaptan[1-3] a selective competitive vasopressin receptor 2 antagonist, the first and only oral drug in its class, is used to treat Hyponatremia (low blood sodium levels) associated with congestive heart failure, cirrhosis, and the syndrome of inappropriate ant diuretic hormone(SIADH). High levels of vasopressin can cause an imbalance that result in low sodium levels and fluid retention. Tolvaptan reduces the level of a vasopressin, and prevents vasopressin-induced re-absorption of water, by competitively blocking vasopressin binding at V2 receptors of distal portions of nephron, promoting aquaresis or electrolyte-free removal of water leading to an increase in urine volume with minimal change in the concentration of electrolytes. Chemically Tolvaptan is (±) -4'-[(7-chloro-2,3,4,5-tetrahydro-5-hydroxy-1H-1-benzazepin -1-yl) carbonyl] -o tolu-m-toluidide. It is not official in any pharmacopoeia; few liquid chromatography procedures have been reported for the determination of Tolvaptan[4-7].

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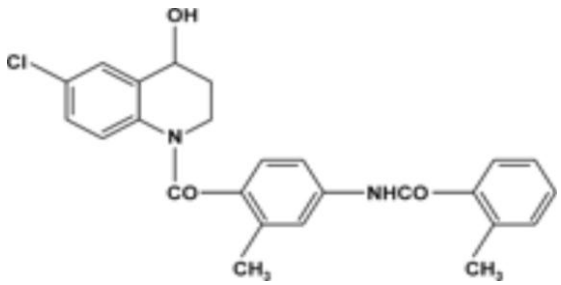


Figure 1 Chemical structure of Tolvaptan

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Samples of tolvpatan was procured from Bio Leo.lab.Pvt.Ltd, Hyderabad, sodium dihydrogen phosphate, (AR grade), Acetonitrile(HPLC Grade) were purchased from Merck Ltd., Worli, Mumbai, India. Tablet formulation (Tolvat) was purchased from the local market.

EQUIPMENT

The HPLC system used for method development and method validation was Waters HPLC e 2695 consisting of auto sampler and UV-Vis Detector with PDA. The output signal was monitored and processed using Empower 2 software.

CHROMATOGRAPHIC CONDITIONS

The chromatographic column used was C18 Nucleosil, 0.01M sodium dihydrogen phosphate and acetonitrile in the ratio of 60:40 was used as mobile phase. Prior to use the solvent was filtered through a 0.45 µ membrane filter and sonicated, flow rate of 0.6ml/min was maintained. The column temperature was maintained at 45^0c and wavelength was monitored at 269 nm (Figure 2).

Preparation of mobile phase

Prepare 0.01M NaH_2PO_4 using HPLC grade water(0.312gm dissolved in 200ml water).Buffer solution was then mixed with acetonitrile in the ration of 60:40.Sonicate the resulting solution and degas using 0.45µ membrane filter.

Preparation of standard solution

The standard stock solution of Tolvaptan was prepared by accurately weighing 15mg of drug and transfer into a 50ml volumetric flask. To this add few ml of diluent and sonicate to dissolve the drug completely. Finally volume is made up to the mark by adding diluent. From the standard stock pipette out 10ml of the solution and transfer in to a 100 ml volumetric flask. Add few ml of diluent and sonicate, finally make up to the volume using the same to obtain 30µg/ml concentration of Tolvaptan. Further the resulting solution was filtered through 0.45µ membrane filter.

Preparation of sample solution

An accurate quantity of powder equivalent to 15mg of Tolvaptan was weighed and transferred to a 50ml volumetric flask. To this add 25 ml of diluent. sonicate to dissolve for 10 min and dilute to volume with diluent.Further filter the resulting solution through membrane filter. From the stock slution 10 ml was taken in a 100 ml volumetric flask and diluted with methanol and sonicate. This secondary stock sample solution was diluted quantitatively with diluent to obtain suitable working sample solutions for chromatographic measurements.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The aim of this study was to develop a simple, accurate and precise RP-HPLC method for the analysis of Tolvaptan in bulk and tablet dosage form using mobile phase and commonly employed Nucleosil C18 column with PDA detector at 269 nm. The typical chromatogram of Tolvaptan was shown in figure 3.The optimal retention time found to be 3.055 min.

METHOD VALIDATION

The aim of method validation was to confirm that the present method was suitable for its intended purpose as prescribed in ICH guidelines[8,9] Q2(R1)

Linearity

Linearity of a detector response for Tolvaptan was demonstrated by preparing solution of Tolvaptan standard over the range of 25%-150% of targeted concentration (dosage). Chromatograms were

recorded and the corresponding retention times and peak areas were listed in table 1.A linear relationship between concentration and area was observed in the linearity range, calibration curve was plotted and shown in figure 4.The correlation coefficient was found to be 1.

Precision

The repeatability(system precision) of the method was checked by repeated analysis of the formulation for six times with the same concentration. Method precision was done by injecting six repeated injections of the standard with different dilutions. The amount of drug present in the formulation was calculated. Precision data is reported in table 2. The %RSD of the peak area of six replicate injections was found to be 0.773%, and for the method precision studies %RSD was found to be 0.024%. The values of % RSD below 2 % indicate that the method was precise.

Accuracy

Accuracy of the test method is demonstrated by %recovery studies performed by spiking sample preparation with known amount of standard at three different concentration levels (80%,100% and 120% of final concentration).Samples were prepared by mixing placebo with Tolvaptan equivalent to about target concentration. Triplicates of sample for each spike level were injected and assay was performed as per the test method. From this "% Recovery" and "% RSD" were calculated. Global recovery result is enlisted in table 3. The mean recoveries were found in the range of 99.74-99.87% which indicates that the method is accurate.

Specificity

The specificity of the test method was demonstrated by studying the interferences from blank, placebo. The blank, placebo and sample solutions were prepared, injected along with the standard preparation and observed for any interference from the blank, placebo and sample solutions at retention time of analyte peak. The chromatograms were identical with nearly same retention time; specificity was confirmed by peak purity. Specificity chromatogram is shown in figure 5.

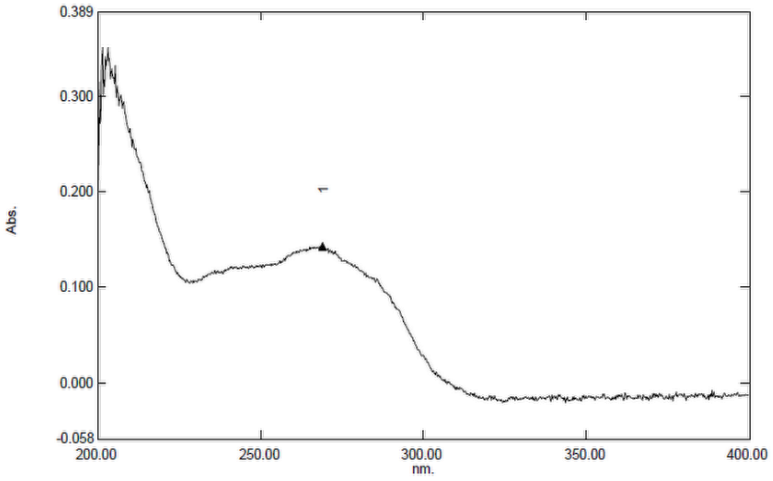
Robustness

Robustness is a measure of capacity of an analytical procedure to remain unaffected by deliberate variations in method conditions. Robustness of a test method is demonstrated by carrying out intentional changes in the mobile phase flow, mobile phase composition and column oven temperature. The sample was analyzed separately by slight changes in the analytical method.

Typical variations included under Validation programme were,

- Flow rate 0.6ml/min ± 0.2ml/min
- Column temperature 45^0c ± 5^0C.

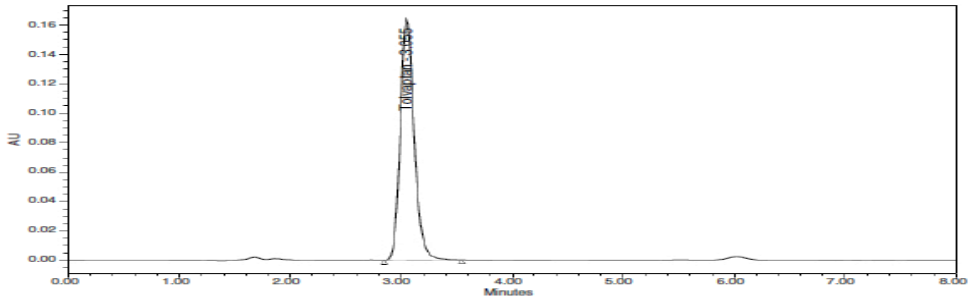
The data for robustness studies is reported in table 4.



No.	Wavelength	Absorbance	Description
1	269.20	0.142	Tolvaptan

Fig 2 :UV Spectrum for Tolvaptan

Fig 3: Chromatogram of standard solution of Tolvaptan.



	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Tailing
1	Tolvaptan	3.055	1429685	164613	100.00	3793.54	1.11

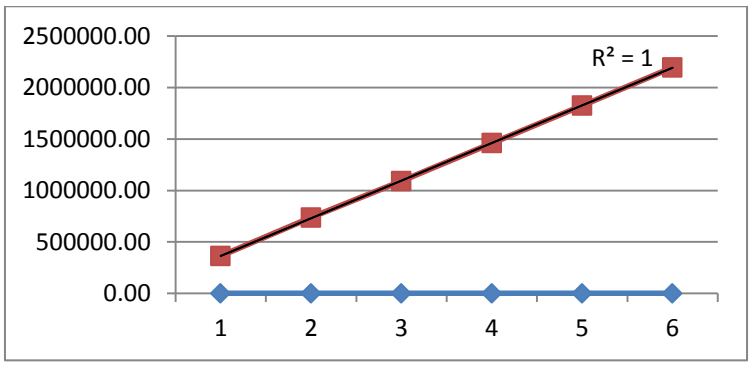


Figure 4 Calibration Curve

TABLE 1: LINEARITY DATA FOR TOLVAPTAN

S.No	%Linearity	Pipetted from stock (ml)	Diluted to volume with diluents(ml)	Concentration(µg/ml)	Peak area
1	25%	2.5	100	7.5	363874
2	50%	5.0	100	15	736990
3	75%	7.5	100	22.5	1090734
4	100%	10	100	30	1459123
5	125%	12.5	100	37.5	2194100
6	150%	15.0	100	45	2194100

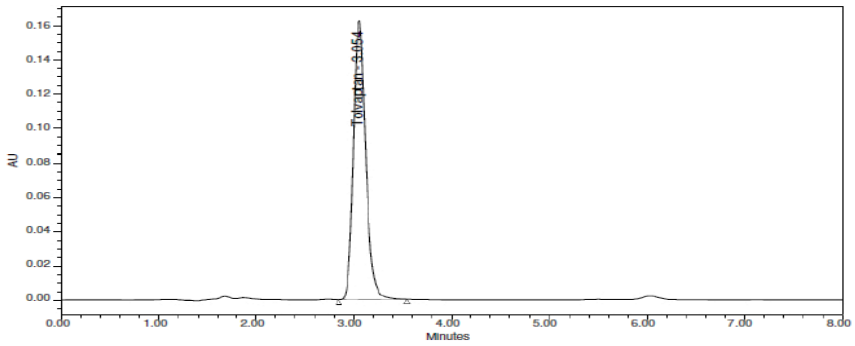
TABLE 2: PRECISION RESULTS FOR TOLVAPTAN

S. No	System precision		Method precision	
	RT	Area	RT	Area
1	3.060	1421964	3.061	1421985
2	3.051	1425199	3.062	1421745
3	3.054	1429690	3.059	1421634
4	3.052	1431655	3.062	1421345
5	3.076	1449901	3.058	1421286
6	3.069	1444792	3.064	1421274
AVG	3.0603	1433867	3.0610	1421545
SD	0.0102	11095.95	0.0022	290.53
%RSD	**0.3324**	**0.7738**	**0.0716**	**0.0204**

TABLE 3: RECOVERY STUDIES OF TOLVAPTAN BY RP-HPLC METHOD:

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
		1150722	24	24.24	101.01		
1	80%	1120634	24	23.808	99.2	99.87	0.993
		1140823	24	23.856	99.4		
		1420422	30	29.949	99.83		
2	100%	1421065	30	30.069	100.23	99.74	0.542
		1418255	30	29.748	99.16		
		1714552	36	35.83	99.54		
3	120%	1721144	36	36.216	100.6	99.83	0.674
		1680741	36	35.766	99.35		

FIGURE 5: SPECIFICITY CHROMATOGRAM OF TOLVAPTAN



	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Tailing
1	Tolvaptan	3.054	1422235	163522	100.00	3685.31	1.12

Table 4 :ROBUSTNESS STUDIES OF TOLVAPTAN

Condition	Variation	Retention time(min)	%RSD
		3.342	
Flow rate	-0.2ml/min	3.302	0.85
		2.777	
	+0.2ml/mim	2.756	0.35
		3.056	
Temperature	-5^0C	3.051	0.11
		3.030	
	$+5^0$C	3.041	0.25

CONCLUSION

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of Tolvaptan in bulk and tablet dosage form. Hence, this method can easily and conveniently adopted for routine analysis of Tolvaptan in pure and its tablet dosage form.

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REFERENCES

[1] Australian public assessment report for Tolvaptan: 2012.
[2] USFDA: Drug safety communications: 2013.
[3] European medicines agency (emeA): CHMP assessment report for Tolvaptan: 2009.
[4] Chaudhari BG, Patel C. Development and Validation of UV -Spectrophotometric Method for the Estimation of Tolvaptan in Bulk and Tablet Dosage Form International Journal for Pharmaceutical Research Scholars-2012, Vol-1(3).
[5] S.Murugan, V.Rajasekharreddy, P. Sirisha, N. Pravallika, K.Chandrakala Method Development and Validation of Tolvaptan in Bulk and Tablet Dosage Form by RP-HPLC Method. International Journal of Research In Pharmaceutical and Nano Sciences-2013, Vol-2(1), P.No-135- 139.
[6] Chakravarthy VK, Gowrishankar D. Development and Validation of RP-LC Method for Estimation of Tolvaptan in Bulk and Its Pharmaceutical Formulation, Rasayan J. Chem-2011, Vol-4(1), P.No-165-171.
[7] Murugan S, Pavan Kumar N, Kiran Kumar C, Syam Sundhar V, Harika S and Anusha P. Method. Development and Validation for Dissolution Method of Tolvaptan in Bulk and Tablet Dosage Form by UV - Spectrophotometry, Indian Journal of Pharmaceutical Science & Research-2013,Vol-3(1), P.No-17-19.
[8] Validation of Analytical procedures text and methodology Q2 (R1).
[9] ICH:Validation of analytical procedures: Methodology Q2B:1996

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