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METHOD DEVELOPMENT AND VALIDATION OF TOLVAPTAN IN ITS API AND FORMULATION BY USING PDA DETECTOR- RP-HPLC

Gogulamudi Anusha*, R. Kalaichelvi

Department of Pharmaceutical Analysis &Quality Assurance

K. C. Reddy Institute of Pharmaceutical Sciences, Medikondur, Guntur, Andhra Pradesh.

Abstract

The present proposed RP- HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of Tolvaptan (TPT) in bulk and pharmaceutical dosage form. The separation was achieved on RP-HPLC with using a PDA detector by incorporation of empower2 software with the flow rate 1 mL/min using Buffer: Acetonitrile (45:55 %V/V) as mobile phase in the column Nucleosil-C_{18} (250 x 4.6 mm, with 5µm) at a wavelength of 264 nm with a run time of 10 mins. The retention time was found to be 4.536 min and data was linear at 25- 150 µg/mL for TVT successively. The Limit of Detection (LOD) and Limit of Quantification (LOQ) was 0.025 and 0.075 respectively for TVT. The percentage recoveries for tolvaptan was found to be 101.28% and the method developed and validated in terms of accuracy, precision, linearity, range, robustness, LOD, LOQ are according to ICH guidelines. So, the method can be useful for the day to day routine analysis in the quality control departments of bulk and pharmaceutical formulations industries.

Keywords: *Tolvaptan, PDA (PhotoDiode Array), ICH and RP-HPLC.*

Address for Correspondence:
R. Kalaichevi, M.Pharm (Ph. D),
Associate Professor,
rkselvi1983@gmail.com

INTRODUCTION:

Tolvaptan[1-5] (TVT), is a selective, competitive vasopressin receptor 2 antagonist used to treat hyponatremia (low blood sodium levels) associated with congestive heart failure, cirrhosis, and the syndrome of inappropriate antidiuretic hormone (SIADH). It is chemically known as *N*-(4-{[(5*R*)-7-chloro-5-hydroxy-2, 3, 4, 5-tetrahydro-1*H*-1-benzazepin-1-yl]carbonyl}-3-methylphenyl)-2-methylbenzamide structurally shown in **fig 1** and its empirical formula is $C_{26}H_{25}ClN_2O_3$ **.** It consists of Molecular weight 448.94 and present in a white to off white crystalline powder and soluble in benzyl alcohol and methanol, practically insoluble in water and melting point was approximately 224°c. Its works by counteracts the actions of vasopressin by blocking the V_2 receptor, thereby decreasing the expression of the aquaporin channels. This causes an increase in free water clearance, a decrease in urine osmolality and an increase in serum sodium concentration. A very few liquid chromatography procedures have been reported for the determination of Tolvaptan[6-8]. So, the present proposed method developed and validated by using RP-HPLC method would serve as a simple, rapid, accurate, reliable and precised method for the determination of Tolvaptan in Bulk and pharmaceutical dosage forms.

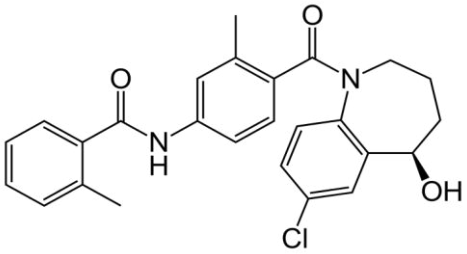

Fig. 1: Structure of Tolvaptan (TVT)

MATERIALS AND METHODS:

Instrumentation:

The method development and validation was done by using Waters 2996 model with a photo diode array (PDA) detector with the column Nucleosil-C_{18} (250 x 4.6 mm, with 5µm).

Chemicals & Reagents:

Tolvaptan gift sample obtained from Centaur pharmaceuticals, Hyderabad, HPLC grade water, Acetonitrile-HPLC, Methanol-HPLC, Sodium dihydrogen phosphste bought from Merck, Mumbai and Ortho-Phosphoric acid from Thermo Fischer Scientific India Private Ltd, Mumbai, India.

Mobile phase preparation:

Buffer and acetonitrile were mixed in the ratio of 10:90 % V/V and sonicated for 20 min.

Buffer Preparation:

Weigh 2 g of sodium dihydrogen phosphate was transferred in to 1000 mL (10 mM) of HPLC water and pH was adjusted to 3.0 with dilute phosphoric acid, then filtered through 0.45 µm nylon membrane filter and sonicated for 20 min.

Preparation of standard solution:

Weigh accurately 10 mg of tolvaptan was weighed and transferred in to 10 mL volumetric flask, 5 mL of mobile phase was added and shaked for 10 min, then the volume was made up to 10 mL with mobile phase and sonicated for 20 min. 0.5 mL of above solution was pipette out and transferred in to 10 mL volumetric flask, then the volume was made up to 10 mL with mobile phase, then filtered through 0.45 µm membrane filter.

Preparation of sample solution:

20 tablets of tolvaptan were powdered by using mortor and pistle. 10mg equilvalent of powdered tablet was weighed and transfered in to 10 mL volumetric flask and 4-5 mL of mobile phase was added, flask was shaked for 15min and the volume was made up to 10 mL with mobile phase, solution was filtered. 0.5 mL of above solution was pippeted in to 10 mL volumetric flask and the volume was made up to 10mL with mobile phase, filtered through 0.45 µm membrane filter.

Chromatographic Conditions:

The column Nucleosil-C_{18} (250 x 4.6 mm, with 5µm) at an ambient temperature with a flow rate of 1.0 mL/min. The mobile phase pumps the sample injection of 10µL. The PDA detector was set at a wavelength of 264 nm for the detection of sample for analysis for a run time of 10 min.

RESULTS AND DISCUSSION:

Method Development:

The method developed was validated as per ICH guidelines. The system suitability parameters are performed. The other validation parameters such as precision, accuracy, linearity, Robustness and LOD &LOQ are also performed by using the optimized chromatographic conditions and standard chromatograph shown in **Fig 2.**

System Suitability:

A standard solution (50 ppm) was prepared by using tolvaptan stock solution as per test method and was injected for five times into the HPLC system. From the system suitability studies, it was observed that % RSD for retention times was 0.04 and for peak areas was 0.02. % RSD values were within the acceptance criteria (limit is ≤ 2 %).

Specificity:

Solutions of standard and sample were prepared as per the test method and injected into chromatographic system.

Precision:

Prepared six standard solutions individually as per test method and injected each solution in to chromatographic system.

Accuracy:

Aliquots of standard drug assay was performed in triplicate as per test method with equivalent amount of tolvaptan into each volumetric flask, for each

spike level to get the concentration of tolvaptan equivalent to 50%, 100% and 150% of the labeled amount as per the test method. Mean % recovery values at each spike level were within the acceptable limit (limit is 98-102%). Accuracy data was presented in **table 1.**

Linearity:
A series of solutions were prepared using tolvaptan working standard solution at concentration levels from 25- 150 µg/mL of target concentration and measured the peak area response of all solutions. A graph was plotted against the concentration (µg/mL) on X-axis *versus* response/Area on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. Linearity graph is shown in **Fig.3.** Linearity results of the method are presented in **Table 2.**

Robustness:
A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected in to the HPLC system using flow rates 0.8 $mL.min^{-1}$, 1.0 $mL.min^{-1}$ and 1.2 $mL.min^{-1}$. The analytical method passed robustness test with well satisfied % RSD. Robustness data was presented in **table 3.**

Limit of Detection (LOD) & limit of Quantification (LOQ):
From the statistical analysis of linearity plot, LOD and LOQ values were calculated and the detection limit and quantitation limit were found to be 0.025 and 0.075 respectively.

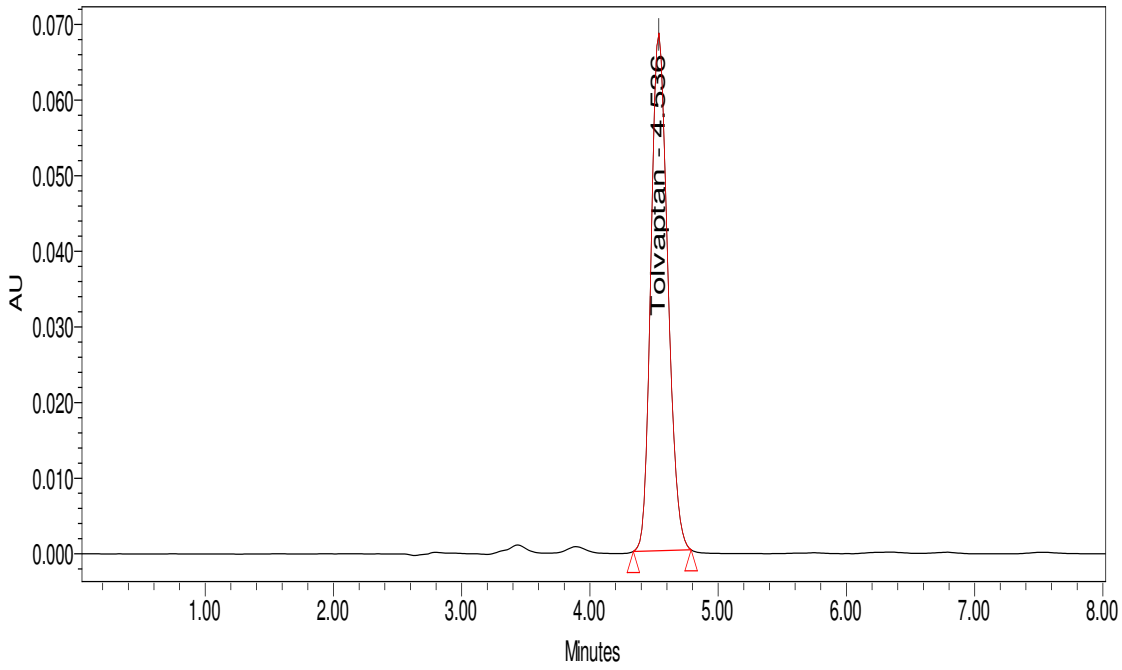

Fig. 2: Typical chromatogram of Tolvaptan (TVT)

Table 1: Data of accuracy of Tolvaptan (TVT)

Concentration-50 ppm (% of spiked level)	Amount found (ppm)	Amount added (ppm)	% Recovery	Statistical analysis of % recovery	
50% standard 1	24.98	25	99.92	Mean	99.18
50% standard 2	24.52	25	98.06	SD	1.04
50% standard 3	24.89	25	99.56	% RSD	1.05
100% standard 1	50.47	50	100.94	Mean	101.24
100% standard 2	50.45	50	100.90	SD	1.15
100% standard 3	50.95	50	101.90	% RSD	1.13
150% standard 1	76.03	75	101.37	Mean	101.18
150% standard 2	75.78	75	101.04	SD	0.17
150% standard 3	75.86	75	101.14	% RSD	0.16

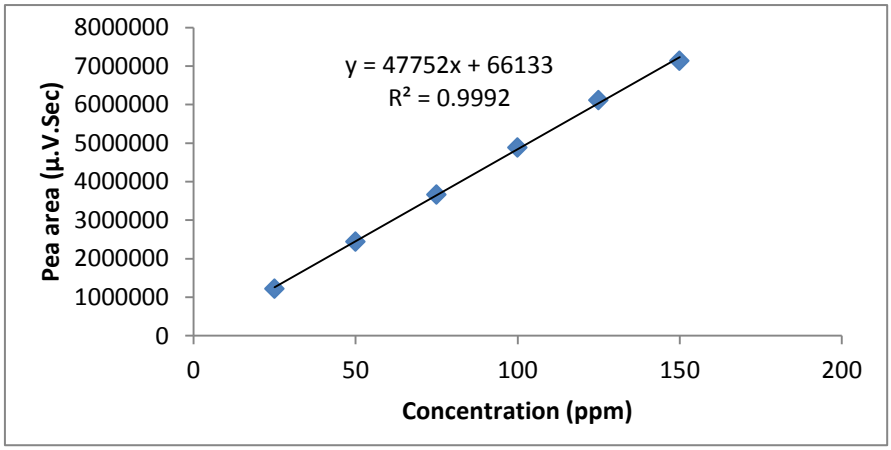

Fig. 3: Linearity curve of Tolvaptan

Table 2: Data of linearity of Tolvaptan (TVT)

Concentration (ppm)	Peak area (µ.V.Sec)	Retention time (min)	No.of theoretical plates (N)	Tailing factor (T.F)
25	1222189	4.48	10324	1.10
50	2443736	4.54	10469	1.11
75	3667176	4.52	10212	1.11
100	4887421	4.54	10198	1.12
125	6113059	4.53	10312	1.11
150	7133211	4.52	10276	1.11

Table 3: Data of robustness of Tolvaptan (TVT)

Factors	Level	Retention time (min)	Tailing factor (T.F)	Theoretical plates (N)
A. Flow rate (mL.min^{-1})	0.8	4.59	1.10	10360.00
	1.0	4.53	1.11	10196.00
	1.2	4.46	1.08	10020.00
Mean		4.53	1.09	10192.00
S.D		0.07	0.02	170.03
% RSD		1.44	1.44	1.66

CONCLUSION:

The present proposed RP-HPLC method using photo diode array (PDA) detector was a simple, accurate, linear, precise and reliable method for the estimation for the Tolvaptan (TVT) can perform the routine analysis in the bulk and pharmaceutical formulation industries in the day to day life as per ICH guidelines with accurate results.

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