STABILTY INDICATING ANALYTICAL METHOD DEVELOPMENET AND VALIDATION FOR ESTIMATION OF TOLVAPTAN IN BULK AND TABLET DOSAGE FORM BY HPLC AND UV

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ABSTRACT

To develop and validate a new, simple, rapid, precise and accurate to quantify Tolvaptan in raw material and dosage forms using C18 analytical column. Mobile phase consisted acetonitrile: water (45: 55 v/v), pumped at flow rate of 1.0 ml/min at ambient temperature and the run time was about 10 min with symmetrical peak. Tolvaptan detected by ultraviolet absorbance at 266 nm with no interference of commonly used excipients. The method was linear over the concentration range 5- 25 mcg/ml. the LOD and LOQ of Tolvaptan were 1.2845mcg/ml and 3.893mcg/ml. the result obtained showed good agreement with the declared contents in case of dosage forms.

Keywords: - *Tolvaptan, HPLC, UV, Method Development, Validation*.

1. INTRODUCTION

Chemically (±) -4- [(7-chloro-2, 3, 4, 5 tetra hydro-5- hydooxy-1H-1-benzazpin-1-yl) carbonyl] -0 tolu-*m*toluidide. Chemical structure of tolvaptan is shown in the fig-1 [1]. Tolvaptan is a selective arginine vasopressin (AVP) V2 receptor blocker used to free water diuresis in treatment of hyper volemic hypernatremia. it appear to be safe and effective at promoting aquaresis and raising serum sodium level in both short and long term studies [2,3]. Tolvaptan also effective for the treatment of congestive heart failure (CHF), but weather there are long standing beneficial effects on CHF is still controversial. Prolonged used of Tolvaptan leads to increase endogenous levels of AVP. Theoretically, this activation could lead to increase after load and cardiac myocite fibrosis, causing the progression of CHF.in addition Tolvaptan is metabolized by the CYP3A4 (human cytochrome P450 3A4) system. Thus physician should be aware of the potential increased interaction with other medications. [4,5]. Literature survey reveals five UV spectrophotometer, HPLC, UPLC,LCMS, method for compound estimation in bulk and pharmaceutical dosage form [6,7].

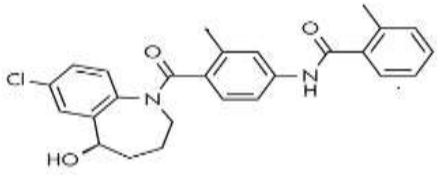

Fig-1. Tolvaptan

2. MATERIAL AND METHODS

2.1 Instruments and apparatus

Shimadzu HPLC system (LC-20AD Multi-solvent delivery system, SPD-20A, UV-Visible Detector, LC solution software). UV-Visible Spectrophotometer (Shimadzu- 1800 double beam, with UV Probe 2.33). Labman sonicator was used for sonication of the sample solution. Thermo scientific pH meter was used to measure pH. A vacuum pump filter was used for the filtration of mobile phase solvents.

Chemicals

The pure drug sample of tolvaptan was procured from Hetero Drug Limited and the tablet dosage form was purchased from Roop Agencies, Pharmaceutical Distribution. SLS (Sodium Lauryl Sulphate) was purchased from Research Lab fine Chem Industry, Mumbai. Acetonitrile and HPLC grade water was procured from Termosil Fine Chem Industry, Nashik, India.

2.2 Chromatographic Condition

The isocratic Mobile phase consisted acetonitrile: water (45: 55 v/v), pumped at flow rate of 1.0 ml/min at ambient temperature and the run time was about 10 min with symmetrical peak. Tolvaptan detected by ultraviolet absorbance at 266 nm with no interference of commonly used excipients.

3. PREPARATION OF SOLUTION

3.1 Preparation of standard stock solution

appropriate weight of 100 mg tolvaptan was transferred in to 100 ml of volumetric flask containing 50 ml of SLS 4%w/v solution sonicated for 15 min and volume made up to 100 ml with the same solvent to an obtain concentration 1000µg/ ml.

A fixed volume of 10 ml of the above solution (1000µg/ml) was transferred into a 100 ml volumetric flask and the volume was made up to 100 ml with the same solvent (SLS 4%w/v) to obtain a concentration of 100µg/ml. the working standard was prepared by the dilution of the standard stock solution[8,9].

3.2 Preparation of Stock Solutions Preparation of standard stock solution

33 mg of tolvaptan was transferred to 100 ml volumetric flask and about 70 ml of acetonitrile was added to it and sonicated to dissolve the contents. The contents was dilutedwith acetonitrile up to the mark and mixed well.

Preparation of standard solution

Transfer 5.0 ml of the standard stock solution into a 100 ml volumetric flask, dilute to volume with dissolution medium and mix.

4. METHOD VALIDATION

The developed method was validated as per the ICH guidelines in terms of linearity, precision, accuracy, repeatability, and stability studies.

a) Linearity

Linearity was assessed by measuring several analyte concentrations varying quantities of standard stock solution was diluted with the SLS (4% w/v) solution to give a 5, 10, 15, 20, and 25 µg/ml concentration. The calibration curve was obtained by plotting the absorbance against concentration (µg/ml) [10].

b) Precision

Precision studies were carried out to ascertain the reproducibility of the developed method. An inter-day precision study was carried out by preparing a drug solution of three different concentrations (10, 15, and 20 µgm/ml of tolvaptan) and analyzing it at three different times in a day. The intraday precision study was carried out by preparing a drug solution of three different concentrations (10, 15, and 20 µg/ml of tolvaptan) and analyzing it on three different days [11].

c) Accuracy

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in a pre-analyzed sample solution of 4 µg/ml. To pre-analyzed sample solution, a known amount of working standard solution of tolvaptan (0.33, 0.42, and 0.48 ml of 100 µg/ml) was added in 10 ml volumetric flask and made up to the mark with diluent which was at different level i.e. 80%, 100%, and 120%. The solutions were analyzed by the proposed method. The mean % recovery from peak areas obtained was calculated [12].

d) Repeatability

Repeatability was determined by preparing six replicates of 15 µgm/ml of tolvaptan and the absorbance was measured at 266 nm [13].

e) Limit of Detection (LOD) and Limit of Quantification (LOQ)

1) LOD

The LOD was estimated from the set of five calibration curves used to determine method linearity. The calibration curve was repeated for 6 times and the SD of the intercept was calculated then LOD was calculated as follow:

LOD= (3.3*SD)/slope.

Where,

SD= the standard deviation of the y-intercept of 5 calibration curves.

Slope= the mean slope of the 5 calibration curves.

2) LOQ

The LOQ was estimated from the set of five calibration curves used to determine method linearity. The LOQ may be calculated as

$LOQ = 10 \times (\sigma/S)$.

Where,

σ = Standard deviation of the Y- intercepts of the five calibration curves.

S = Mean slope of the five calibration curves [14-16]

5. RESULT AND DISCUSSION

5.1. Chromatographic Conditions

Column:	C18
Mobile Phase:	Acetonitrile: water (45:55)
Flow Rate:	1.0 ml/min
Detection Wavelength:	266 nm
Run time:	10 min
Injection volume:	20.0 µL

5.2. HPLC method validation

a) System suitability

The system suitability was performed by injecting standard solution containing 200µg/ml of tolvaptan in six replicates. For two of them, the peak asymmetric were 2000 and %RSD of tolvaptan was less than 2. The result indicates that the system suitability parameter was within the acceptable limit. The result are illustrated in table 4.1.

Table-4.1 the system suitability results

Parameter	Results
Theoretical plates per column	Not less than 2000
Symmetric factor/tailing factor	Not more than 2
Retention time	3-5 minutes

b) Linearity

The linearity for tolvaptan were assessed by analysis of standard solution in range of 35-175 µgm/ml and 0.7,1.4,2.1,2.8, and 3.5 ml of solutions were pipette out from the Stock solution of tolvaptan (500 µgm/ml) and transferred to 10 ml volumetric flask and make up with diluent to obtain 35,70,105,140, and 175 µgm/ml. The linearity for tolvaptan were assessed by analysis of standard solution in range of 35-175 µgm/ml. Correlation co-efficient for calibration curve tolvaptan was found to be 0.997. The calibration curve of tolvaptan is depicted in fig-2.

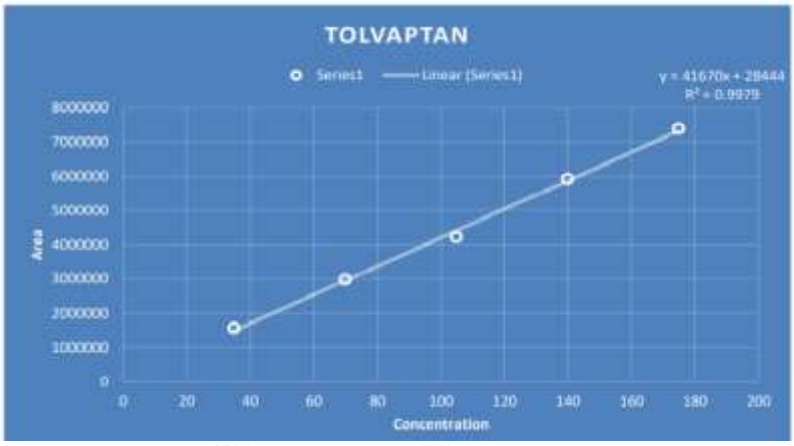

Fig-2: The calibration curve of tolvaptan [35-175 µgm/ml]

Table-4.2 The peak area, mean, SD, and % RSD of tolvaptan obtained in linearity

Sr. No	Conc. (µgm/ml)	Area	Mean	SD*	% RSD**
1	35	1529816	1552155	19502.26	1.26
		1565789			
		1560859			
2	70	2942480	2966969	39522.64	1.33
		3012564			
		2945863			
3	105	4240682	4217426	56852.56	1.34
		4152632			
		4258963			
4	140	5862497	5909046	54047.48	0.91
		5896321			
		5968321			
5	175	7486518	7373362	126376.9	1.71
		7396584			
		7236985			

*SD= standard deviation **%RSD= relative standard deviation

c) Precision

The precision of the developed method has been checked by studying it on interday and intraday. The precision data has been tabulated in table 4.3. Standard solution containing (70, 140, 210 µgm/ml) of tolvaptan were analyzed three times on the same day and on the different three days and % R.S.D was calculated.

Table-4.3 The precision data of tolvaptan

Sr. No.	Conc. (µgm/ml)	Area		II	III	Mean	SD*	% RSD*
	Intraday							
1	70	3105499		3005499	3105500	3005499.00	57735.32	1.92
2	140	5788060		5688060	5788062	5754727.33	57735.60	1.00
3	210	8757192		8657192	8757195	8723859.67	57735.89	0.66
	Interday							
1	70	2949671		2949675	2849671	2949675.00	57736.18	1.96

2	140	5778196		5678196	5778199	5744863.67	57735.89	1.01
3	210	8523602		8423602	8523605	8490269.67	57735.89	0.68

*SD= standard deviation **%RSD= relative standard deviation

d) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Table-4.5 The LOD and LOQ data of tolvaptan

Parameters	Result
SD of intercept	59260.368
Slope	41670
LOD(µg/ml)	4.693
LOQ(µg/ml)	14.221

e) Accuracy

100 µgm/ml drug solution was taken in three different flask label A, B and C. Spiked 50%,100% and 150% of standard solution in it and diluted up to 10 ml. The area of each solution peak was measured at 266 nm. The amount of tolvaptan was calculated at each level and % recoveries were computed. The accuracy data of tolvaptan is tabulated in table 4.6

Table-4.6 The accuracy data of tolvaptan

% conc.	Sample amount (µgm/ml)	Amount added (µgm/ml)	Amount recovered (µgm/ml)	% recovery	% Recovery mean	S.D.	% RSD
50%	12.5	7.5	7.56	100.8	99.90	0.870	0.871
	12.5	7.5	7.49	99.86			
	12.5	7.5	7.43	99.06			
100%	20	15	14.92	99.46	100.13	1.64	1.63
	20	15	15.3	102			
	20	15	14.84	98.93			
150%	27.5	22.5	22.3	99.11	100.5	1..31	1.31
	27.5	22.5	22.7	100.66			
	27.5	22.5	22.89	101.73			

f) Robustness

For robustness study, the following parameters has been changed one by one and their effects on system suitability was observed.

i) Change mobile phase composition by ± 1.0 mL of organic solvent.

ii) Change Wavelength ± 1 nm

iii) Change flow rate ±0.1 mL/min.

Change in mobile phase composition: Std. working solution was injected three times by change in the mobile phase composition by ±1.0 mL of organic solvent (Acetonitrile: water) (44.11v/v and 56.09v/v) of developed method.

Change in wavelength: Std. working solution was injected three times by change in the Wavelength by ± 1 nm of sample (265 nm and 267 nm) of developed method. Calculate the% RSD of mean area for change in method parameter.

Change in flow rate: Std. working solution was injected three times by change in the flowrate by ± 0.1 mL/min (0.9 mL/min and 1.1mL/min) of developed method. Calculate the %RSD of mean area for change in method parameter. The robustness data of tolvaptan istabulated in table 4.7.

Table-4.7 The robustness data of tolvaptan

Condition	Peak area mean	SD	% RSD
Change in ratio of mobile phase ± 1ml	5962632	24880.24	0.42
	6058536	73565.62	1.21
Change in wavelength ± 1nm	5975503	78830.27	1.31
	6065253	67944.48	1.12
Change in flow rate ± 1ml	5920746	58860.81	0.99
	6125365	46099.98	0.75

g) Repeatability

Standard solution containing tolvaptan (35 µgm/ml) was injected six times and areas of peaks were measured and % RSD was calculated. The repeatability data of tolvaptan is tabulated in table 4.8.

Table-4.8. The repeatability data of tolvaptan

Sr. No.	Conc. (µgm/ml)	Area	Mean	SD	%RSD
1	35	1529816	1561062	16148.4	1.03445
2	35	1530789			
3	35	1529865			
4	35	1528845			
5	35	1530852			
6	35	1529856			

h) Stability of the Solution

The stability of analytical solution was verified by analyzing the standard and filtered sample solution initially and also at different time intervals as mentioned below by storing in sample compartment of HPLC instrument at ambient condition. The calculated cumulative percentage RSD for peak areas of tolvaptan for both the sample and standard solution are tabulated in table 4.9. Accurately weighed 10mg of tolvaptan drug was taken in 10 ml of volumetric flask and then diluted with diluent up to mark [1000 µgm/ml] and sonicated for 15 min. 1 ml of this solution was transferred in 10 ml volumetric flask and diluted up to with diluent [100 µgm/ml]. The chromatogram obtained in stability of tolvaptan is given in fig.

Table-4.9. The stability data of standard and sample solution of tolvaptan

Time in hours	Peak area mean	SD	% RSD
Standard Solution Stability			
0	5984535	44906.96	0.75
8	6014970	59722.31	0.99
16	5993785	60527.62	1.00
24	5951909	17509.31	0.29

Sample Solution Stability			
0	6047600	13964.18	0.23
8	6048632	15681.7	0.26
16	6066662	32638.11	0.53
24	6060293	23501.03	0.39

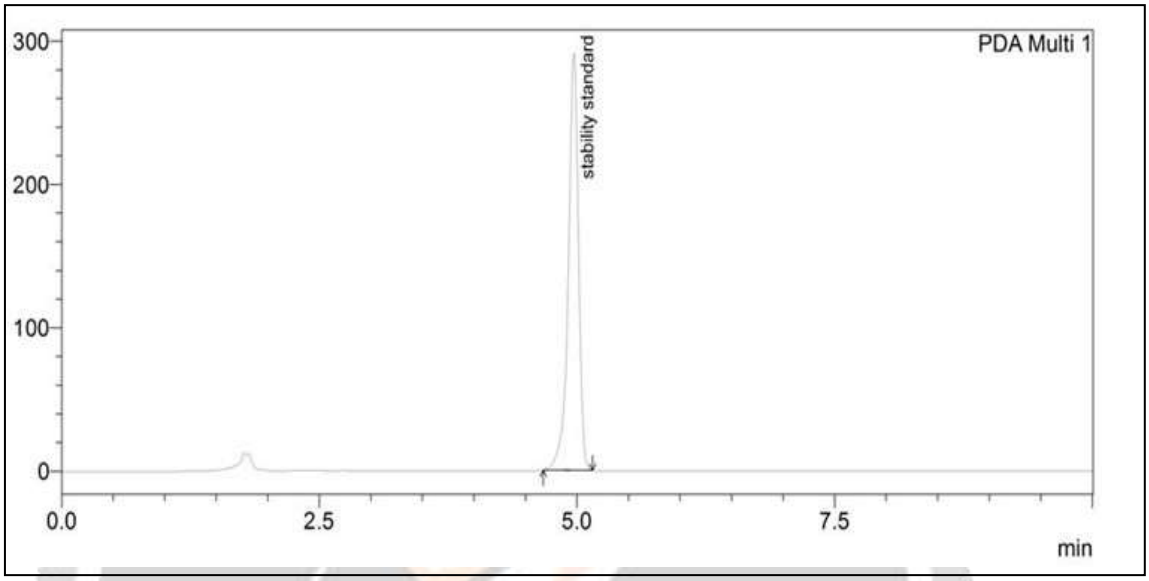

Fig-3 The chromatogram of standard tolvaptan obtained in stability studies (area=3511068)

6. CONCLUSION

The proposed method was found to be simple, accurate, and precise and HPLC method suitable for the estimation of Tolvaptan in bulk and pharmaceutical dosage forms. All the parameters meet the standard of ICH guidelines for method validation and found to be simple, sensitive, accurate and precise .The method was developed by selecting and detection wavelength of 266nm. Mobile phase acetonitrile: water (45:55).The optimized chromatographic condition were a C18 column with run time 10 minutes. The developed HPLC method has been successfully applied for the quantitative estimation of Tolvaptan in the commercial tablet formulations. From the above result it was concluded that the developed UV and HPLC method are precise, and accurate and can be applied for the quantitative estimation of Tolvaptan from bulk and tablet dosage form, the method can be used for the routine testing of Tolvaptan by the Pharmaceutical industry.

7. ACKNOWLEDGMENT

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