RESEARCH ARTICLE

Spectrophotometric Method and Its Validation for Tolvaptan in Its Bulk and Marketed Formulation Including Stress Studies

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ABSTRACT

Tolvaptan in API and formulation may now be quantified using a technique. The solution was diluted with acetonitrile and scanned in the UV area between 200 and 400 nm. At 267 nm, tolvaptan exhibits maximum absorption. The accuracy investigations had been executed at3 levels, i.e., 80, 100, and 120%, and recuperation turned into discovered to bewith inside the variety of 99.4% for the tolvaptan, which showed linearity over the range of 5 to 160 g/mL with correlation co-efficient (r2) of 0.9995. The quantification (LoQ) and detection (LoD) threshold were 0.471 and 1.435 g/mL, respectively. The suggested approach underwent forced deterioration, and each parameter's degradation was discovered. The ICH rules were followed in the validation of each parameter.

Keywords: Acetonitrile, Tolvaptan, UV-visible spectrophotometer.

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INTRODUCTION

An antagonist of the vasopressin receptor, tolvaptan is a drug.[1-5] Treatment for both eUVolemic and hypervolemic conditions. Hyponatremia (low blood sodium levels)is linked to congenital heart failure (CHF), chlorosis, and the syndrome of inadequate antidiuretic hormone (SIADH).[6-12] Lower blood sodium serum levels and fluid retention can result from an increase in vasopressin. Inhibiting vasopressin's function in the nephron's collecting tube, tolvaptan binds to vasopressin receptors.[13-16] When molecules, atoms, or ions in a sample transition from a lower energy state to a higher energy state in the UV area (200–400), spectroscopy measures the amount of EMR radiation that is received or emitted. It operates according to Beer's-Law. Lambert'sStudies on forced degradation are done to determine whether a medicine is stable or breaks down under stressful circumstances. Solid-state degradation under forced conditions (heat, heat, humidity, and light).[17] The drug profile is shown in Table 1.

MATERIALS AND CHEMICALS REQUIRED

Apparatus: Volumetric flask, pipette, beakers.
Chemicals: Tolvaptan, acetonitrile.

Instrument: Double beam UV-visible spectrophotometer (ELICO SL210).

METHOD DEVELOPMENT

Selection of Solvent

A sequence of trails was done to determine the solvent for dissolving the drug. The solvents such as water, methanol and acetonitrile, sodium lauryl sulphate, DSMO. The drug was found to be freely soluble in acetonitrile, insoluble in water.[9,10] Acetonitrile was selected as optimized solvent for estimation of tolvaptan by a UV-visible spectrophotometer.

Preparation of Standard Solution

Weigh approximately 5 mg of tolvaptan in 5 mL volumetric flask and dissolve upto the mark with acetonitrile to give 1000 µg/mL from the 1000 µg/mL pipette out 1-mL into a 10 mL volumetric flask dilute upto the mark to give 100 µ/mL.

Determination of λ_{max}

Take 1-mL from 100 µg/mL into 10 mLvolumetric flask make upto the mark with acetonitrile and scanned in UV-vis spectrophotometer from 200–400 nm. Tolvaptan shows maximum absorption at 267 nm. It was shown in Figure 1.

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METHOD VALIDATION PARAMETERS

Linearity

Linearity is a method determined by taking absorbance and concentration to now weather the concentration increases with absorbance.[11-13]

Preparation of Standard Solution

Tolvaptan 5 mg was weighed, transferred to a 5 mL flask, and then dissolved in acetonitrile to provide a 1000 µg/mL concentration. One mL of the aforementioned solution was transferred to a 10 mL volumetric flask using acetonitrile as volume builder 100 µg/mL are produced. We used the same stock solutions to prepare successive dilutions.

preparation of Working Solutions from Stock

A series of working solutions were made by transferring varying aliquots of the tolvaptan standard solution (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 0.5 g/mL) to 10 mL volumetric flasks, and volume was created with ACN up to 5–160 g/mL of solutions, respectively. The method for tolvaptan was linear for the concentration range of 5–160 g/mL. Table 2 contains the results of the calculation for the correlation coefficient, Yintercept, and slope of the regression line.

Precision

Six replicates of standard solution carry out precision was checked for absorbance values (n = 6) of tolvaptan (50 µg/mL) without changing the parameters for the method. And %RSD was caluculated as shown in (Table 3). %RSD should be less than 2.

Accuracy

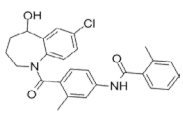
Accuracy was performed at three levels: 80, 100, 120% and the %RSD was caluculated and it is shown in Table 4.

Procedure

Preparation of Standard Solution

Tolvaptan, 5 mg, in a 5 mL volumetric flask, diluted to a 1000 µg/mL concentration with acetonitrile. 100 µg/mL can be obtained by pipping off 1-mL of this 1000 µg/mL solution into a 10 mL volumetric flask. Pipette the working solution from 0.5, 1, 2, 3, 4, and 5 to 5, 10, 20, 30, and 40 µg/mL.

Table 1: Drug profile

IUPAC name	*N-[4-(7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1-benzazepine-1-carbonyl)-3-methylphenyl]-2-methylbenzamide*
Chemical formula	$C_{26}H_{25}ClN_2O_3$
Molecular wt	448.9 g/mole
Pka	13.84
Melting point	225–230°C
Boiling point	594.4°C
Solubility	Freely soluble in methanol, acetonitrile Sparingly soluble in water
Structure	

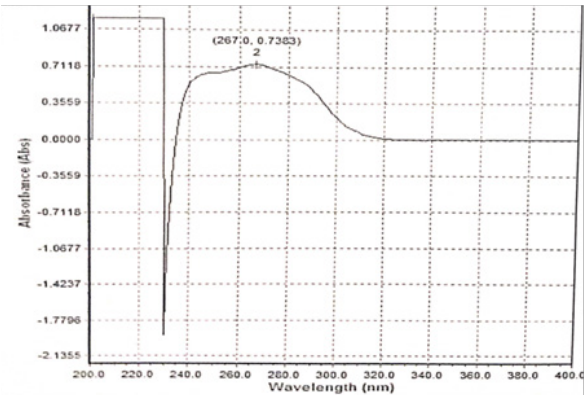


Figure 1: λ_{max} of Tolvaptan.

Preparation of Sample

10 tablets of tolvaptan was weighed and average weight (83.3 mg) of tablets was taken and powdered. Weigh about 54.6 mg, equivalent to 10 mg and transfer to 10 mL volumetric flask to give 1000 µg/mL and this solution was sonicated for 10 minutes and filtered.

Pipette 0.5 mL of the aforementioned solution to yield 50 g/mL, and then acetonitrile dilute to the desired concentration.

Table 2: Linearity of tolvaptan

Concentration(µg/mL)	*Absorbance*
5	0.092
10	0.132
15	0.272
20	0.401
25	0.512
30	0.621
35	0.742
40	0.858
45	0.986
50	1.138
60	1.258
70	1.386
80	1.523
90	1.672
100	1.828
110	1.952
120	2.112
130	2.212
140	2.342
150	2.452
160	2.556

Table 3: Precision of Tolvaptan

Concentration (µg/mL)	*Absorbance*
50	0.624
50	0.623
50	0.623
50	0.620
50	0.624
50	0.620
Mean	0.622333
SD	0.00186
%RSD	0.299%

Table 4: Accuracy of Tolvaptan

Concentration (µg/mL)	*Absorbance*	*%Recovery*	*Mean Recovery (%)*
80% (50+40)	1.024	94.46%	93.73
	1.022	92.28%	
	1.024	94.46%	
100% (50+50)	1.204	99.3%	99.26
	1.206	99.5%	
	1.201	99.0%	
120 (50+60)	1.398	98.1%	97.95
	1.396	98.03%	
	1.392	97.72%	

Table 5: Robustness of Tolvaptan

Concentration (µg/mL)	*266 nm*	*267 nm*	*268 nm*
50	0.521	0.523	0.624
50	0.519	0.521	0.621
50	0.521	0.522	0.620
50	0.520	0.520	0.623
50	0.522	0.521	0.622
50	0.524	0.622	0.621
Mean	0.62033	0.6215	0.62183
SD	0.00136	0.00104	0.00147
%RSD	0.2202%	0.1687%	0.2367%

Table 6: Ruggedness of Tolvaptan

Concentartion (µg/mL)	*Instrument -1*	*Instrument -2*
50	0.633	0.621
50	0.622	0.624
50	0.621	0.620
50	0.620	0.622
50	0.621	0.623
50	0.622	0.621
Mean	0.6215	0.62183
SD	0.001049	0.0011472
%RSD	0.1687%	0.2367%

Table 7: LoD and LoQ of Tolvaptan

Drug	*LoD (µg/mL)*	*LoQ (µg/mL)*
Tolvaptan	0.471	1.435

Spiking Procedure

The accuracy was tested by adding sample solution to standard solution at three different concentrations: 80, 100, and 120%. A standard amount of 80, 100, and 120% is added to the sample.

LoD and LoQ

LoD and LoQ were determined by the slope of the regression equation. Calculated by the signal-to-noise ratio.

$$LoD = 3.3 \times \sigma/S$$

$$LoQ = 10 \times \sigma/S$$

Robustness

Robustness is the evaluation of an analytical method where even small change in the parameters should not vary in results.

Procedure

six aliquots of 50 µg/mL of standard solution was scanned at the wavelength (±) 1 nm and (±) 2 nm of fixed wavelength and calculated the %RSD, mean, standard deviation. As shown in Table 4.

Ruggedness

The test results obtained should not be changed even if it is performed in different conditionssuch as different analysts and instruments (Table 5).

Assay of Pharmaceutical Formulation

Preparation of Standard Stock Solution

Tolvaptan created through taking 5 mg in a 5 mLvolumetric flask and diluting with acetonitrile to acquire a 1000 µg/mL concentration. Pipette 1-mL from a 1000 µg/mL answer into a 10 mL volumetric flask to get 100 µg/mL. Pipette 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 40 to 50 µg/mL of the concentration.

Preparation of Sample

A total of 10 tablets of tolvaptan turned into weigh and average eweight (83.3 mg) of tablets turned into takenand powdered. Weigh a powder equivalent weight (54.6 mg) to 10 mg and switch to 10 mL volumetric flask to provide 1000 µg/mL. And the answer turned into sonicated for 10 minutes and filtered. 0.5 mL of filterate turned into taken to provide 50 µg/mL and dilute to the mark with acetonitrile.

Forced Degradation Studies

%Degradation of Tolvaptan was shown in Figure 3.

Acid Hydrolysis

Add 1-mL of ^{1}N HCL and depart the aggregate to face for three hours after including 1-mL of well known concentration. then diluted to the attention of 10 µg/mL and neutralized with ^{1}N NaOH. in ultraviolet spectroscopy and scanned.

Base Hydrolysis

To make 10 µg/mL of tolvaptan, blend 1-mL of known concentration with 1-mL of 0.1 N NaOH answer for three hours, then neutralize with ^{1}N-HCl.

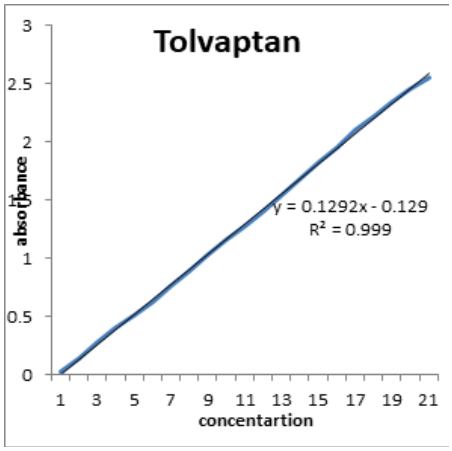


Figure 2: Calibration curve of Tolvaptan.

Peroxide Hydrolysis

In 1-mL an aliquot from the same old inventory answer and 1 mL of 30% hydrogen peroxide had been used to dilute tolvaptan to a very last attention of 10 µg/mL.After that, the treatment changed into allowed to face for 6 hours.[17,18]

Heat Degradation

An oven changed to warmness 15 mg of a pattern among 45 and 70°C. This pattern need to be used to create a 1000 µg/mL answer. The quantity changed into then adjusted with diluent to the right quantity after 1-mL of the aforementioned answer changed into taken and transferred to a 10 mL volumetric flask. We scanned this answer at 267 nm.

Bench Top Hydrolysis

Following the switch of one mL from the inventory answer into a 10 mL volumetric flask and the essential degree of dilution with diluent, this answer changed into scanned at 267 nm at initial, 24 and 48 hours.

RESULTS AND DISCUSSION

Linearity

Absorbance was directly proportional to concentration. Calibration curve was shown in Figure 2. All concentration gave linear absorbance and regression coefficient value r^2 was found to be 0.999.

Precision

%RSD was found to be less than 2. Hence the method was found to be precise.

Accuracy

%recovery was calculated. The %recovery of tolvaptan should be within 90 to 102%. The results are shown in Table 3.

Robustness

%RSD was found to be less than 2. The result of robustness was shown in Table 5.

Ruggedness

%RSD was found to be less than 2. The result of robustness was shown in Table 6.

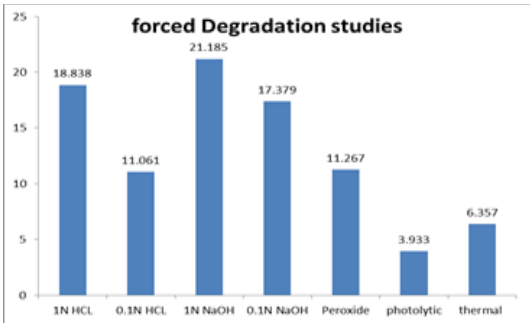


Figure 3: %Degradation of Tolvaptan.

LoD and LoQ

Results of LoD and LoQ was shown in Table 7.

Assay

%Recovery of tolvaptan was found by two methods.

Compendial Method

Sample absorbance is 0.592.
Standard Absorbance = 0.620
Standard concentration = 50
The sample concentration is equal to the product of the sample's absorbance and the standard concentration.

= (0.592/0.602)*50
=49.15

%Assay = (sample absorbance/standard absorbance) x (standard concentration/sample concentration).
=(0.592/0.602)*(49.15/50)x 100
=0.983 x0.983x100
= 96.6 %

Y-Intercept Method

Y=mx + c
0592=0.128X+0.128
Y= 47.6

% Assay =(observed absorbance/original absorbance) x 100
=(47.6/50) x 100=95%

CONCLUSION

By studying all the parameters the defined approach displaying linear reaction within side the variety 5160 µg/mL for tolvaptan. The information of pharmaceutical formulation is notably reproducible and reliable. And drug balance take a look at suggests that there's considerable degradation observed in stress situation of tolvaptan. Hence, the approach may be used for the recurring analysis of tolvaptan in tablet dosage form.

REFERENCES

1. Chakravarthy VK, Gowrishankar D. Development and validation of RP-HPLC method for estimation of tolvaptan in bulk and its Pharmaceutical formulation.Rasayan J. Chem. 2011;4(1):165-171.
2. Toshiki M, Hiroyuki F, Yoshitaka Y, Shigeki N, Toyoki M. Tolvaptan, an Orally Active Vasopressin V2-Receptor Antagonist-Pharmacology and Clinical Trials. Cardiovas. Drugs rev.2007;25: 0-13.
3. Hauptman PJ, Zimmer C, Udelson J, Shoaf SE, Mallikaarjun S, Bramer SL, Orlandi C. Comparison of two doses

anddosing regimens of tolvaptan in congestive heart failure. J CardiovascPharmacol.2005;46(5):609-14.
4. Sojeong Yi, Hyewonjeon, Sea hyunyoon, Joo-youncho, Sanggoo shin, In-jinjang, KyUVg-sang yu. Pharmacokineticsand pharmacodynamics of oral tolvaptan administered in 15-60mg single doses to korean men. J Cardiovasc Pharmacol,59(4), 2002, 315-22.
5. Pratil MR, Patil AS, ShirkhedkarAA. Novel and ecofriendly UV-Spectrophotometry methods for estimation of tolvaptan using hydrotropic agent. Int J Pharm Chem Anal.2019;6(4):115- 119.
6. Murugan S, Kumar NP, Kumar CK, Sundhar VS, HarikaS,Anusha P et al. Method development and validation for dissolution method of tolvaptan in bulk and tablet dosageform by UV spectrophotometry. Indian J Pharm Sci Res 2013;3(1):17-9.
7. Murugan S, Pavan Kumar N, Kiran Kumar C, SyamSundar 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 and research. 2013;3(1):17-19.
8. V. KalyanChakravarthy, D. Gowri Shankar. Development and validation of RP – HPLC method for estimation of tolvaptan in bulk and its pharmaceutical formulation. Rasayan J. Chem. 2011;4(1):1165-171.
9. Lanka A. Ramprasad, J.V.L.N.S Rao, SrinivasuPamidi, Vara Prasad J, Naga Raju D. Impurity profiling of tolvaptan tablets using new stability indicating UPLC method. International Research Journal of Pharmacy.2012 ;3(11):21-25
10. Pei.Q,Bikui Zhang, Hongyi Tan, LihuaLiv, MiLuo; Development and validation of an LC-MS/MS method for the determination of tolvaptan in human plasma and its application to a pharmacokinetic study. Journal of Chromatography. B. 2013;913(914):84-89
11. Shoa f SE, Wa ng Z, Br icmont P, Mallikarjun.Pharmacokinetics, Pharmacodynamics,and Safety of Tolvaptan, a NonpeptideAVPAntagonist, During Ascending Single Dose Studies in Healthy Subjects". J. Clin. Pharmacol. 2007;47:1498-1507.
12. Erences Hauptman PJ, Zimmer C, Udelson J, ShoafSE,Mallikaarjun S, Bramer SL. Comparison of two dosesand dosing regimens of tolvaptan in congestive heartfailure. J Cardiovasc Pharm. 2005;46(5):609-614.
13. Gandhi BM, Rao AL, Rao JV. A New Stability-Indicating and Validated RPHPLC Method for the Estimation of Tolvaptan in Bulk and Pharmaceutical Dosage Forms.Asian J. Pharm. Anal. 2017;12(2):31-44.
14. Rao BV, Sowjanya GN, Ajitha A, Rao VUM. A review on stability-indicating HPLC method development. World J Pharm Pharm Sci. 2015;4(08):405-423.
15. Smela JW. Regulatory considerations for stability indicating analytical methods in drug substance and drug product testing. Am Pharm Rev.1980;8(3): 51-54.
16. Carstensen T, Hong D, Shah M. Development and Validation of HPLC stability-indicating assays. Drug stability, 3rd ed. New York: Marel Dekker Inc. 2008; 3rd ed:364-366.
17. Carstensen T, Hong D, Shah M. Regulatory Aspects of Stability Testing in Europe. Drug stability, 3rd ed. New York: Marel Dekker Inc.2008;3rd ed.:594-596.