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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TOLVAPTAN IN BULK AND ITS TABLET DOSAGE FORM BY UV- SPECTROPHOTOMETRY

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Abstract:

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Validation is a documented program that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification. The main objective was to develop and validate the UV-spectrophotometric method for the estimation of tolvaptan in bulk and pharmaceutical formulations as per ICH guidelines. The initial stock solution of tolvaptan was prepared in acetonitrile. The λ_{max} of tolvaptan was found to be 269 nm. The drug obeyed Beer-Lambert's law in the concentration range of 20-140% of standard concentration (µg/ml) of 30 mg with coefficient of correlation (r^2) was 0.999. The accuracy studies of proposed method was performed at three different levels, i.e., 50%, 100%, and 150% and recovery was found to be in the range of 100.4%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.34 and 0.94 µg/ml respectively. The %RSD less than 2 which indicates the accuracy and precise of the method. The above method was a rapid tool for routine analysis of tolvaptan in the bulk and in the pharmaceutical dosage form. The method can be useful for the day-to-day routine analysis in the quality control departments of bulk and pharmaceutical formulations industries. So, we concluded that a simple, rapid, precise and accurate spectrophotometric method has been developed for quantitative analysis of tolvaptan API and its tablet formulation.

Key words: *Tolvaptan, UV-Spectrophotometry, vasopressin receptor-2 antagonist, linearity, accuracy, validation.*

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INTRODUCTION:

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Under computer-controlled instrumentation, derivative spectrophotometry is acting a very important role in the single or multicomponent analysis of drugs by UV-molecular absorption spectrophotometric method. Pharmaceutical research is developing increasingly complex molecules and drug formulations, and each novel and highly selective analytical technique is therefore of much potential interest[1].

Molecular absorption in the ultraviolet and visible region of the spectrum is dependent on the electronic structure of the molecule. Absorption of energy is quantized, resulting in the elevation of electrons from orbital in the ground state to higher energy orbital in the excited state. The wavelength range of UV radiation starts at the blue end of the visible light and ends at $2000A^0$. Validation is a documented program that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification[2,3].

Tolvaptan a selective competitive vasopressin receptor-2 antagonist, the first and only oral drug in its class. It is a diuretic agent. It is used to treat Hyponatremia (low blood sodium levels) associated with congestive heart failure (CHF), cirrhosis, and the syndrome of inappropriate anti-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[4,5].

Tolvaptan is a white to off white crystalline powder with a molecular weight 448.94g/mol. Tolvaptan is soluble in benzyl alcohol and methanol, practically insoluble in water and hexane. Tolvaptan melting point was approximately 224 ^{0}C. Its empirical formula is $C_{26}H_{25}ClN_2O_3$. The chemical structure of tolvaptan was illustrated in Figure 1. It is not official in any pharmacopoeia; few liquid chromatography procedures have been reported for the determination of tolvaptan. The author has 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. It is not official in any pharmacopoeia; few liquid chromatography procedures have been reported for the determination of tolvaptan. The newly developed method was proven to be accurate, sensitive and precise. Validation parameters tested included limit of detection, limit of quantitation, linearity, accuracy, precision, specificity and solution stability[6,7].

The present work describes the development and validation of UV-spectrophotometric method, which can quantify the tolvaptan. An attempt was made to develop a simple, accurate, precise and rapid spectrophotometric method for the estimation of tolvaptan in bulk and pharmaceutical dosage form[8]. The method was validated as per International Conference on Harmonization (ICH) guidelines.

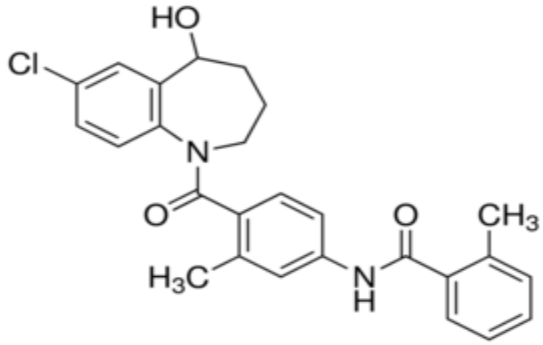

Fig. 1: Molecular structure of tolvaptan

MATERIALS AND METHODS:

Instruments used

The analysis was performed on A double beam UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. And other instruments are Schimadzu Digital Electronic Balance-BL 220H, and Ultrasonic cleaner were purchased from Life Care Equipment Pvt. Ltd., Hyderabad, Telangana, India.

Chemicals and reagents used

The pure API sample of tolvaptan was obtained as free gift sample from KP Labs, Hyderabad, Telangana, India. The marketed pharmaceutical dosage form of tolvaptan tablets (15 mg) was purchased from local Apollo Pharmacy, Hyderabad, Telangana, India. All reagents like HPLC grade methanol, HPLC grade water and HPLC grade acetonitrile are purchased from Hyderabad, Telangana was utilized throughout the experiment.

Selection of solvent
A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and acetonitrile were tried based on the solubility of the drug. Tolvaptan was found to be freely soluble in acetonitrile, insoluble in water and methanol. Acetonitrile was selected as optimized solvent in this spectrophotometric method.

Preparation of standard stock solution
An accurately weighed quantity of tolvaptan 50 mg was transferred to 50 ml of clean and dried volumetric flask, dissolved in 20 ml, the final volume was made with acetonitrile to obtain standard solution having concentration of 1000 µg/ml. 1 ml of this solution was transferred to 10 ml volumetric flask, volume was made with acetonitrile, it gives 100 µg/ml. These stock solutions were used to prepare further dilutions throughout the experiment.

Selection of wavelength (λ_{max})
Appropriate volume 0.1 ml of standard stock solution of tolvaptan was transferred into a 10 ml volumetric flask, diluted to a mark with acetonitrile to give concentration of 1 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Analytical method validation developed
The aim of method validation was to confirm that the present method was suitable for its intended purpose as prescribed in ICH guidelines. The method was validated in order to determine the linearity, precision, accuracy, repeatability, ruggedness, LOD and LOQ of the method.

Linearity
Linearity and range different concentrations of tolvaptan solutions were prepared. The range of the solutions varies from 20% to 140% of standard concentration (µg/ml) of 30 mg. The absorbance of these solutions is noted. The absorbance of the lower-level linearity solution (20%) and the higher-level linearity solution (140%) in 6 replicates were recorded. The graph of concentration vs absorbance of linearity solutions was plotted.

Repeatability
Repeatability was determined by preparing six replicates of 1 µg/ml of tolvaptan and the absorbance was measured at 280 nm.

Precision
Precision studies were carried out to ascertain the reproducibility of the proposed method. Intraday precision study was carried out by preparing drug solution of three different concentrations (0.5, 1 and 2 µg/ml of tolvaptan) and analyzing it at three different times in a day. Interday precision study was carried out by preparing drug solution of three different concentrations (0.5, 1 and 2 µg/ml of tolvaptan) and analyzing it at three different days.

Accuracy
Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the %recovery was calculated.

Limit of Detection and Limit of Quantitation
The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\mathbf{LOD = 3.3 \times \sigma/S}$$
$$\mathbf{LOQ = 10 \times \sigma/S}$$

Where,
σ = Standard deviation of the response, and
S = Slope of the calibration curve.

Ruggedness studies
Ruggedness studies were performed by preparing three replicates of 1 µg/ml of tolvaptan, analysing by two different analyst and on two different instruments and the results are reported as %RSD.

Assay for pharmaceutical formulation
The solution was filtered through Whatman filter paper No. 41. 0.5 ml this solution was transferred to 10 ml volumetric flask and final volume was made with acetonitrile. It gives 0.5 µg/ml. It was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 269 nm against blank solution of acetonitrile. Determine the amount of % tolvaptan in tablet according to the following formula:

$$\%\ \mathbf{Assay} = \frac{\mathbf{WS\ X\ AT\ X\ Sample\ D.F.\ X\ Avg.\ Wt.}}{\mathbf{AS\ X\ Standard\ D.F.\ X\ WT\ X\ LC}}\ \mathbf{X\ PS}$$

Where,
WS = weight of standard;
WT = weight of sample
AT = Absorbance of tolvaptan in the test solution,

AS = Absorbance of tolvaptan in the standard solution,
Standard D.F. = Standard dilution factor,
Sample D.F. = Sample dilution factor,
PS = Purity of working standard [%],
LC = Label claim of tolvaptan.

RESULTS AND DISCUSSION:

Absorbance maxima (λ_{max})

The absorbance maximum of tolvaptan was found to be 269 nm. The UV spectrum for tolvaptan is depicted in Figure 2.

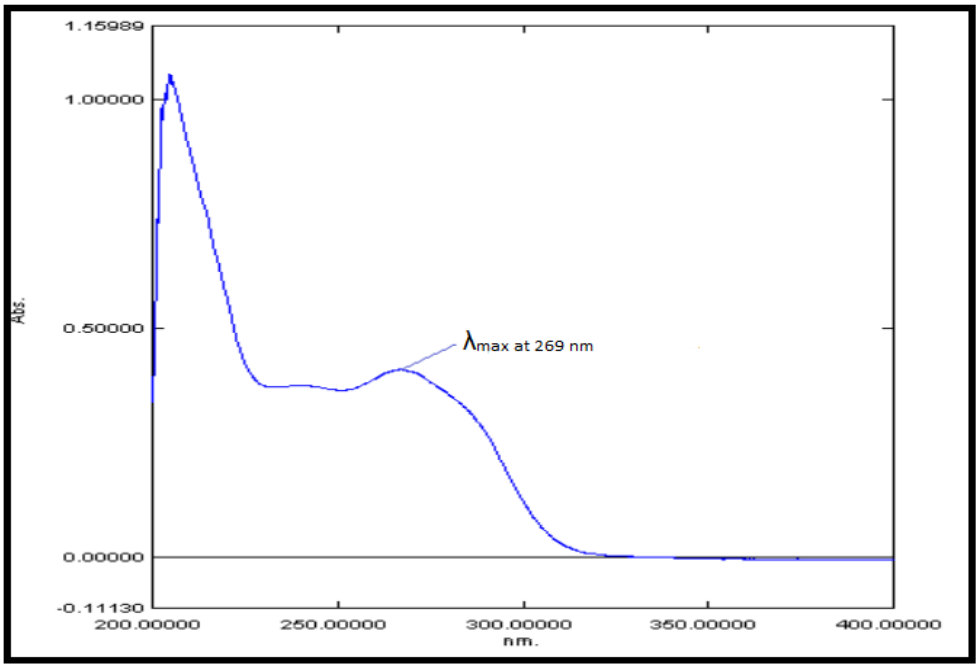

Fig. 2: Absorption spectrum of tolvaptan

Method validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

Linearity

Standard solutions of tolvaptan in the concentration range of 20 to 140% were observed in UV-Spectroscopy.

A graph of absorbance (on Y-axis) versus concentration (on X-axis) was plotted and calibration graph was shown in Figure 3. The regression equation was found to be Y=0.148x+0.075, Correlation coefficient was 0.9998.

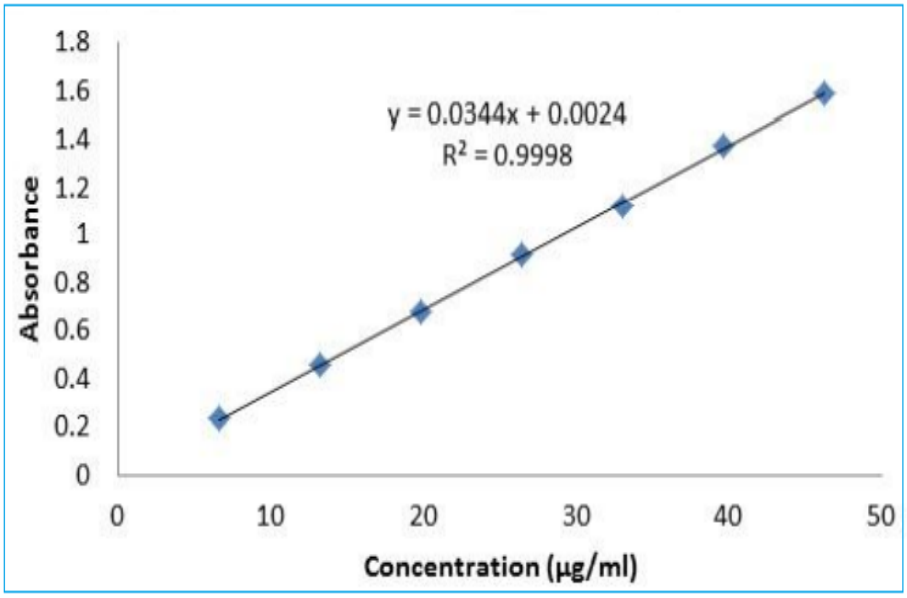

Fig. 3: The graph of concentration vs absorbance of linearity solutions

Repeatability

Repeatability was determined by analyzing 1 µg/ml concentration of tolvaptan for six times with % RSD < 2 which illustrated in Table 1.

Table 1: Repeatability studies

Conc. in µg/ml	Absorbance at 269 nm	Absorbance Mean	SD	%RSD
6	0.642	0.646	0.008	**1.295**
6	0.656			
6	0.650			
6	0.652			
6	0.635			
6	0.638			

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 that indicate this method precise for the determination of the pure form. The inter and intraday precision results were mentioned in Table 2 and 3 respectively.

Table 2: Intraday precision

Conc. in µg/ml	Intraday Precision	%RSD
	Absorbance Mean ± S.D. (n=3)	
2	0.197 ± 0.001	1.015
6	0.626 ± 0.006	0.962
10	1.032 ± 0.004	0.477

Table 3: Interday precision

Conc. in µg/ml	Interday Precision	%RSD
	Absorbance mean± S.D. (n=3)	
2	0.196 ± 0.001	0.510
6	0.627 ± 0.002	0.421
10	1.033 ± 0.003	0.339

Accuracy

Accuracy shall be determined by performing recovery studies at 3 levels in which known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level and the % recovery was calculated. The mean recovery was found between 100-101 % and %RSD between 0.7-1.0. The results are shown in Table 4.

Table 4: Recovery studies

Spiked level (%)	Formulation conc. (µg/ml)	Pure drug conc. (µg/ml)	Amount conc. recovered (µg/ml)	% Recovery	% Mean recovery SD	%RSD
50	2	1	3.01	100.6	99.24 ± 1.311	**0.32**
	2	1	2.97	99.0		
	2	1	2.94	98.0		
100	2	2	4.02	100.7	100.24 ± 0.491	**0.490**
	2	2	3.99	99.7		
	2	2	4.00	100.2		
150	2	3	4.96	99.2	99.74 ± 0.487	**0.488**
	2	3	4.99	99.8		
	2	3	5.00	100.19		

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

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD and LOQ values are 0.34 and 0.94 µg/ml respectively. The results are illustrated in Table 5.

Table 5: LOD and LOQ

Drug	LOD	LOQ
Tolvaptan	0.34 µg/ml	0.94 µg/ml

Ruggedness studies

This study was performed by analyzing 4 µg/ml of tolvaptan by two different analysts and on two instruments, results of the study were given in Table 6 and %RSD obtained was less than two which is within the acceptance limits.

Table 6: Ruggedness of tolvaptan

Parameter	Conc. (µg/ml)	Absorbance	Absorbance Mean ± S.D. (n=3)	%RSD
Different Analyst	4	0.405	0.407±0.0025	**0.61**
		0.41		
		0.407		
Different instrument	4	0.404	0.408±0.0036	**0.88**
		0.409		
		0.411		

Assay for pharmaceutical formulation

The percentage recovery for tolvaptan tablet formulation was found to be 99.6-101.06 % enlisted in Table 7. The results for assay are within acceptable limit.

Table 7: Assay of tolvaptan tablets

Label claim (mg)	Amount found (mg)	% Purity	Mean % purity ± SD (n=3)	%RSD
15	15.07	100.49	100.16±0.788	**0.787**
15	15.10	100.73		
15	14.88	99.26		

Table 8: Summary of validated parameters

Parameters	Method
λ_{max}	269 nm
Beers law limit	1-10 µg/ml
Correlation coefficient (r^2)	0.999
Molar absorptivity	$3.41x10^4$ L mol^{-1} cm^{-2}
Regression equation (y=mx+c)	Y=0.1036x+0.0006
Slope (m)	0.1036
Intercept (c)	0.0006
Accuracy	99.24-100.24
Precision	0.339-1.015
LOD	0.310 µg/ml
LOQ	0.941 µg/ml

CONCLUSION:

The method does not involve any tedious procedural steps; do not require any extra reagents or longer analysis time and a very simple instrument are required. The method can be used to determine the purity of the drug available from various sources. Because of cost-effective and minimal maintenance, the present UV-Spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of tramadol hydrochloride in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable, and is in good agreement with all validation parameters. Hence, the method can be used for the routine analysis of tolvaptan in tablet dosage form. The results obtained on the validation parameters met ICH and USP requirements. The developed method was found to be simple, accurate, precise, linear and having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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Declarations

Author contributions

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information

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