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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AREA UNDER CURVE APPROACHES FOR TOLVAPTAN ESTIMATION IN BULK AND TABLET DOSAGE FORM

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Abstract:

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Tolvaptan in bulk and Tablet dosage form. The drug shows maximum absorption (λmax) at 267nm in Acetonitrile solution and Area under Curve [AUC] in absorption spectra was measured between the wavelengths ranges 262 to 272nm which obeys Beer's law in the concentration range of 3 - 18 µg/ml The linearity study was carried out and regression coefficient was found to be 0.998 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 98.66%,102.4% and 99.05%. The % relative standard deviation (%RSD) was found to be less than 2%. The LOD and LOQ were found to be 0.06µg/ml and 0.184µg/ml. According to ICH guidelines the method has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine Quantitation of Tolvaptan in bulk and pharmaceutical dosage form.

KEYWORDS: *Tolvaptan ;Area Under curve;Validation; RP-HPLC; HPTLC, Anti-Diuretics.*

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INTRODUCTION:

Tolvaptan is non-peptide vasopressin (VP) V2 receptor antagonist that inhibits water re-absorption in the kidney by competitively blocking VP binding, resulting in water diuresis without significantly changing total electrolyte excretion. Tolvaptan chemically is N-{4-[(5R)-7-chloro-5-hydroxy-2,3,4,5 tetrahydro-1H-1-benzazepine-1-carbonyl]-3 methylphenyl}-2-methyl benzamide[1]. Its mechanism of action involves enhancing free water clearance and ameliorating serum sodium levels. Given its therapeutic significance, the development of a sensitive and precise analytical method is imperative for determining Tolvaptan concentrations in pharmaceutical formulations. This medication finds application in the management of hypernatremia (low blood sodium) in individuals with heart failure or syndrome of inappropriate antidiuretic hormone (SIADH). Additionally, Tolvaptan is employed to decelerate the decline of kidney function in adults who face a heightened risk of rapidly progressing autosomal dominant polycystic kidney disease (ADPKD). Tolvaptan binds to V2 receptor with 1.8 times greater affinity than ADH. The drug is highly plasma protein bound (99%). About 40% of tolvaptan is bioavailable and the terminal half-life is about 12 h[2]. Tolvaptan dissolves readily in organic solvents like acetonitrile and ethanol but is essentially insoluble in water. Acetonitrile is frequently chosen as a solvent for UV spectrophotometric measurement because of its solubility properties. Achieving clear spectra, a well-shaped peak, and repeatable absorbance values depends heavily on the choice of solvent.

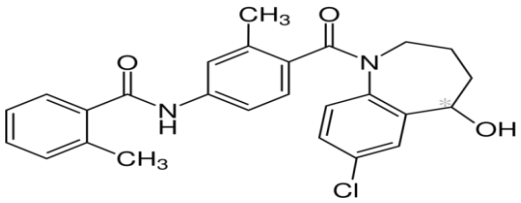

Figure 1: Chemical Structure of Tolvaptan.

Few analytical methods using UV, HPLC, RP-HPLC, and HPTLC have been described for determining Tolvaptan in pure medication and pharmaceutical dosage forms, according to review of the literature. The current endeavor attempts to create and validate a novel, fast, simple, precise, and specific Zero order derivative UV Spectrophotometric method for estimating Tolvaptan in tablet and bulk dose form.

MATERIALS AND METHODS:

Instrument:

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals:

Tolvaptan pure drug gift sample was given by by Medrich Pharmaceuticals Pvt Ltd and its pharmaceutical dosage Tolvaptan 20 tablets obtained from New sky health pharma pvt ltd.

Solvent:

Acetonitrile is used as a solvent.

Selection of analytical wavelength:

Appropriate dilutions of Tolvaptan were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400 nm. The absorption spectra obtained and show maximum absorbance at 262 nm and 272 nm, as the wavelength for detection.

Preparation of standard stock solution:

100mg of Tolvaptan was weighed accurately transferred into 100 ml of volumetric flask and diluted in Acetonitrile upto the mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 ml into 10 ml individual volumetric flask and diluted in Acetonitrile up to the mark, this gives 3, 6, 9, 12, 15 and 18µg/ml concentration.

Preparation of sample solution:

20 tablets of Tolvaptan marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Tolvaptan was transferred into 100ml volumetric flask then it was diluted with Acetonitrile and make upto the mark.

METHOD AND VALIDATION:

The method was validated according to the ICH guidelines.

RESULT AND DISCUSSION:

Method: Zero order derivative spectroscopy

Linearity:

Linearity shows how well the response of the method changes in proportion to the concentration of the drug within a given range. In other words, when the concentration increases, the absorbance should also increase in a consistent and predictable manner. The linearity was established in the range of 3-18µg/ml was measured at 262 nm and 272 nm and absorbance values are shown in table 1. The calibration curve was prepared by plotting graph against the concentration vs absorbance and therefore the graph shown in Fig-3 statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in table-2.

Precision:

Precision indicates how close the results are when the same sample is tested multiple times under similar conditions. If the variation between

repeated measurements is very small, the method is considered precise. Precision was established by intra-day and inter-day was determined by analysing the same concentration for six times in a same day. Inter-day precision was analysing the same concentration daily for six days shown in table-3.

Accuracy:

The accuracy of an analytical method says that closeness of test results obtained by that method of the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels at 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. Shown in table -4.

Ruggedness:

Ruggedness evaluates whether the method gives consistent results when small changes are made, such as using different analysts or performing the test on different days. A rugged method produces similar results despite these minor variations. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (Table-5)

LOD and LOQ:

LOD is the smallest amount of drug that the method can detect, even if it cannot measure it accurately. LOQ is the lowest amount of drug that can be measured accurately and precisely using the developed method. LOD and LOQ were calculated by using following formula

LOD = 3.3(σ/S) and LOQ = 10 (σ / S)

LOD and LOQ value of Tolvaptan were found be 0.06µg/mL and 0.184µg/mL.

Table 1: Results of calibration curve at 267nm by area under curve spectroscopy

Sl No	Concentration in µg|ml	Absorbance ± Standard deviation
1	0	0
2	3	0.150±0.0013
3	6	0.260±0.0013
4	9	0.370±0.0016
5	12	0.506±0.0026
6	15	0.623±0.0032
7	18	0.774±0.0024

** Average of six determinations*

Table 2: Regression parameters of Tolvaptan by area under curve spectroscopy.

Regression	Parameter Results
Range	3-18 µg/ml
λ_{max}	262nm & 272nm
Regression equation	y = 0.0418 x + 0.0068
Slope(b)	0.0418
Intercept (a)	0.0068
Correlation coefficient	0.9988
Sandell's sensitivity	0.024
LOD(µg/ml)	0.06µg/ml
LOQ(µg/ml)	0.184µg/ml

Y=bx+a**

Table 3: Determination of Precision results for Tolvaptan at 267nm by area under curve spectroscopy.

Concentration (µg|ml)	Intra-day Absorbance ± Standard deviation*	% RSD	Inter-day Absorbance ± Standard deviation*	% RSD
3	0.153±0.0011	0.721	0.153±0.0012	0.819
6	0.261±0.0013	0.514	0.267±0.0021	0.798
9	0.370±0.0013	0.363	0.380±0.0022	0.595
12	0.503±0.0017	0.351	0.516±0.0024	0.482
15	0.618±0.0021	0.341	0.629±0.00177	0.281
18	0.773±0.00129	0.167	0.782±0.00208	0.266

** Average of six determinations,** Percentage relative standard deviation.*

Table 4: Determination of accuracy results for Tolvaptan at 267nm by area under curve spectroscopy

Spiked levels	Amount of sample (µg|ml)	Amount of standard (µg|ml)	Amount recovered	Recovery± Standard deviation*	%RSD**
50	9	4.5	13.29	98.49±0.948	0.962
10	9	9	18.45	102.4±0.474	0.462
150	9	13.5	22.28	99.05±0.365	0.336

** Average of six determinations,** Percentage relative standard deviation.*

Table 5: Determination of ruggedness results of Tolvaptan at 267nm by area under curve spectroscopy.

Analysts	Day-1	Day-2
Mean absorbance	0.370	0.380
±Standard deviation*	0.0013	0.0022
%RSD**	0.363	0.595

** Average of six determinations,** Percentage relative standard deviation.*

FIGURES:

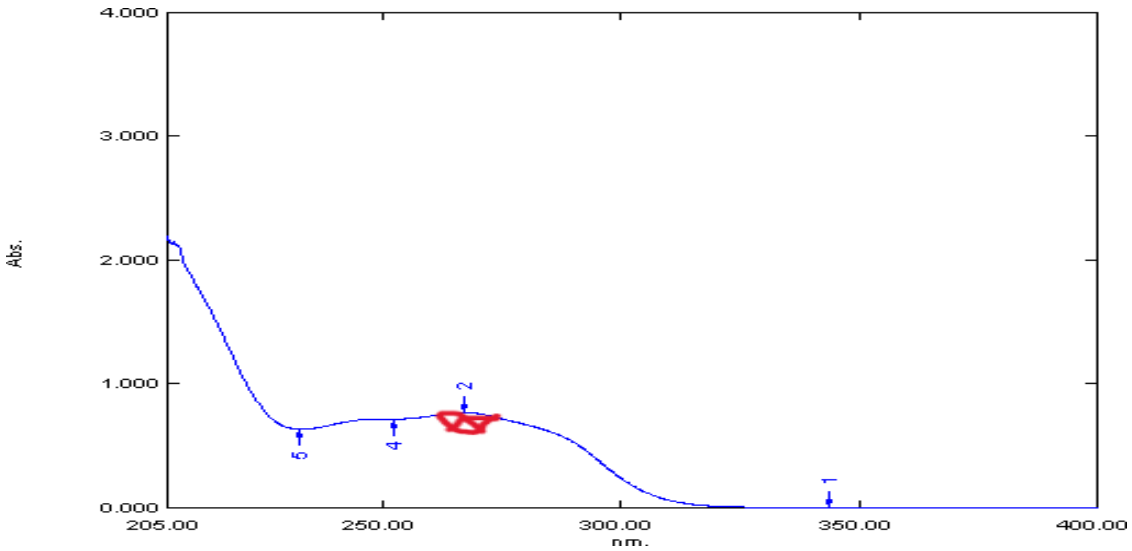

Fig. 2: Area under curve spectrum of Tolvaptan at 262 nm to 272 nm.

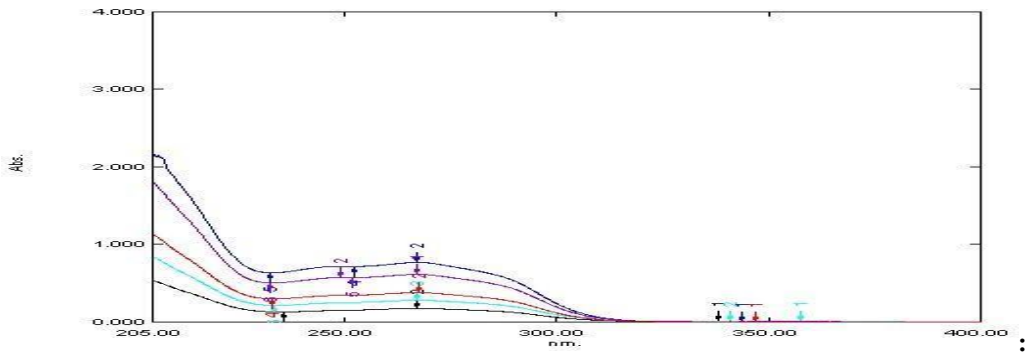

Figure 3: Area under curve overlain spectra of Tolvaptan showing absorbance at 262 nm to 272 nm.

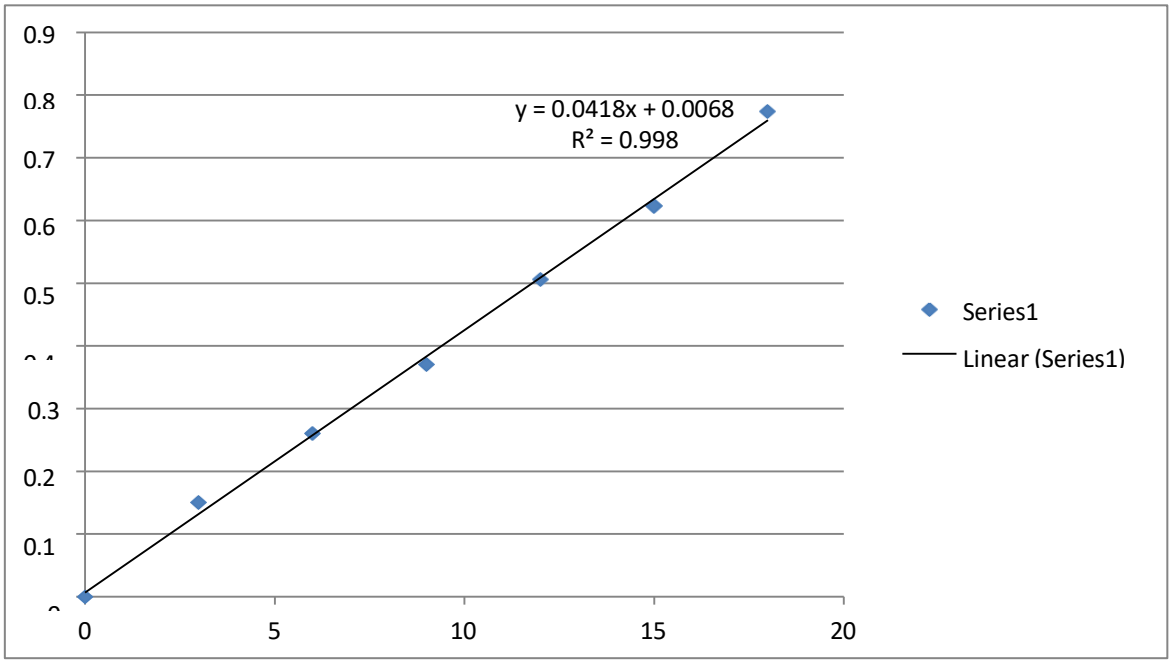

Figure 4: Calibration curve of Tolvaptan by Area under curve.

CONCLUSION:

The analytical method developed for Tolvaptan was validated as per ICH guidelines demonstrating simplicity, specificity, accuracy, economy, and sensitivity. This method is suitable for regular analysis of Tolvaptan in both bulk form and pharmaceutical preparations.

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