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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ZERO-ORDER APPROACHES FOR TOLVAPTAN ESTIMATION IN BULK AND TABLET DOSAGE FORM

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ABSRTACT

A novel, accurate, and efficient zero order derivative UV spectroscopic method has been developed and validated for the quantification of Tolvaptan in both bulk drug and pharmaceutical dosage forms. Tolvaptan exhibits maximum absorbance at 267 nm in Acetonitrile and its concentration follows Beer's Law within the range of 3-18 µg/mL. The method showed strong linearity, with a correlation coefficient (r2) of 0.9967, indicating high consistency and reliability across the studied range. Recovery rates were observed between 98.5%, 103.1% and 98.7%, while the limits of detection (LOD) and quantification (LOQ) were found to be 0.066 µg/mL and 0.202 µg/mL respectively. The method also demonstrated excellent precision, with relative standard deviation (%RSD) values below 2%. All validation parameters-linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ-were assessed according to ICH guidelines. This validated spectroscopic approach proves to be a dependable and reproducible method for routine analysis of Tolvaptan in various pharmaceutical preparations.

KEYWORDS: Tolvaptan Quantification, Validation, RP-HPLC, HPTLC, Anti-diuretics.

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.

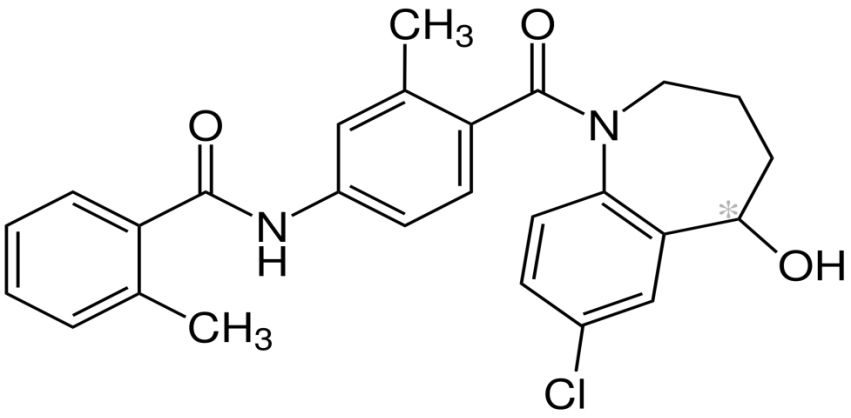

Figure 1: Chemical Structure of Tolvaptan.

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 267 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 267nm 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 0.066µg/mL and 0.202µg/mL.

Table 1: Results of calibration curve at 267nm by zero order derivative spectroscopy.

Sl No	Concentration in µg|ml	Absorbance ± Standard deviation
1	0	0
2	3	0.147
3	6	0.276
4	9	0.373
5	12	0.494
6	15	0.606
7	18	0.736

* Average of six determinations

Table 2: Regression parameters of Tolvaptan by Zero order spectroscopy.

Regression	Parameter Results
Range	3-18 µg/ml
λmax	267nm
Regression equation	y = 0.0408x + 0.013
Slope(b)	0.0408
Intercept (a)	0.013
Correlation coefficient	0.9969
Sandell's sensitivity	0.024
LOD(µg/ml)	0.066
LOQ(µg/ml)	0.202

Y=bx+a**

Table 3: Determination of Precision results for Tolvaptan at 267nm by Zero order spectroscopy.

Concentration (µg|ml)	Intra-day Absorbance ± Standard deviation*	% RSD	Inter-day Absorbance ± Standard deviation*	% RSD
3	0.149± 0.0003	0.27292	0.175 ±0.0005	0.31253
6	0.273± 0.0004	0.18869	0.280 ± 0.0004	0.14532
9	0.369± 0.0004	0.13963	0.377 ± 0.0005	0.14499
12	0.493± 0.0005	0.11093	0.496 ± 0.0005	0.11026
15	0.605± 0.0005	0.09042	0.614 ± 0.0008	0.14555
18	0.766± 0.0004	0.06734	0.767 ± 0.0005	0.06725

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

Table 4: Determination of accuracy results for Tolvaptan at 267nm by Zero order 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	98.50±0.244	0.247
100	9	9	18.57	103.16±0.132	0.127
150	9	13.5	22.23	98.76 ± 0.103	0.104

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

Table 5: Determination of ruggedness results of Tolvaptan at 267nm by Zero order spectroscopy.

Analysts	Day-1	Day-2
Mean absorbance	0.369	0.377
±Standard deviation*	0.0004	0.0005
%RSD**	0.139	0.144

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

FIGURES

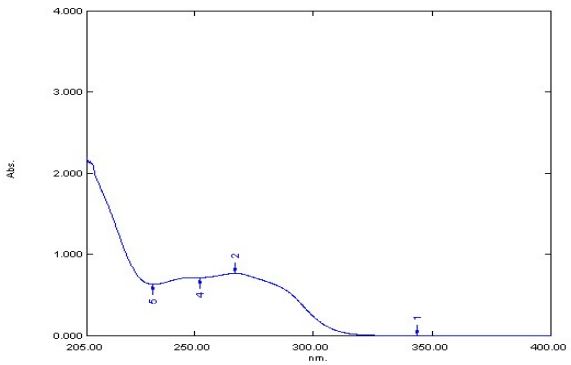

Fig. 2: Zero order spectrum of Tolvaptan at 267nm.

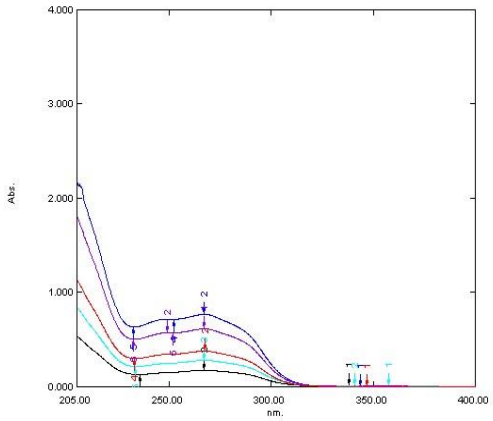

Figure 3: Zero Order Overlain Spectra of Tolvaptan Showing Absorbance At 267nm.

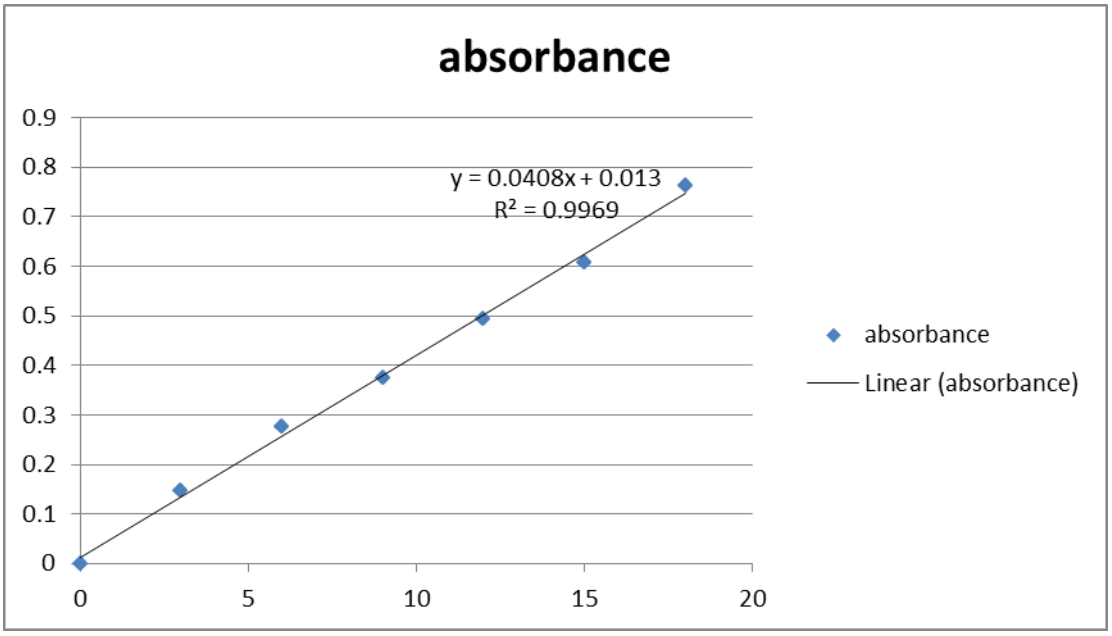

Figure 4: Calibration curve of Tolvaptan by Zero order derivative spectroscopy.

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.

REFERENCE

1. Patil MR, Patil AS, Shirkhedkar AA. Novel and ecofriendly UV-Spectrophotometry methods for estimation of Tolvaptan using hydrotropic agent. International Journal of Pharmaceutical Chemistry and Analysis, 2019; 6(4): 115-9.
2. Kumudini Rahul Pawar, Sanjay K Bais. A Validated bioanalytical method for quantification of Tolvaptan by RPHPLC-UV. Journal of Population Therapeutics & Clinical Pharmacology, 2022; 04: 985-994.
3. Patil MR, Patil AS, Shirkhedkar AA. Novel and ecofriendly UV-Spectrophotometry methods for estimation of Tolvaptan using hydrotropic agent. International Journal of Pharmaceutical Chemistry and Analysis, 2019; 6(4): 115-9.
4. Chakravarthy VK, Shankar DG. Development and validation of RP-HPLC method for estimation of Tolvaptan in bulk and its pharmaceutical formulation. Rasayan J Chem, 2011; 4(1): 1165-71.
5. Gandhi BM, Rao AL, Rao JV. A new stability-indicating and validated RP-HPLC method for the estimation of tolvaptan in bulk and pharmaceutical dosage forms. Asian Journal of Research in Chemistry, 2014 Jul 1; 7(7): 2.

6. Hashmi SA, Alegete P. QbD green analytical procedure for the quantification of tolvaptan by utilizing stability indicating UHPLC method. BMC chemistry, 2024 Jun 28; 18(1): 122.
7. Iram S, Pal S, Murtaja Ali MA, Ahamed M, Akther S. Analytic Method development And validation for the quantitative estimation of Tolvaptan in API form and its marketed pharmaceutical dosage form by RP-HPLC, 2024; 13(2): 30-38.
8. Sutar AS, Magdum CS. Application of response surface methodology in development and optimization of stability indicating RP-HPLC method for determination of tolvaptan in bulk and formulation. Journal of Pharmaceutical Research International, 2021 Oct 12; 33(46A): 137-48.
9. Bhavyasri K, Aishwarya B, Suchitra D, Sumakanth M. Spectrometric Bioanalytical Method Development and Validation of Tolvaptan in Spiked Human plasma Followed by Forced degradation Studies. Research Journal of Pharmacy and Technology, 2023 Dec 1; 16(12): 5996-6001.
10. Pawar KR, Kale PG, Nalawade MS, Chavan SS, Warude BJ, Kaldate DP. Methodological Advancements in Tolvaptan Analysis: HPLC-Based Stability Indication with LC-MS Validation, 2023; 24(4): 334-346.
11. Karadurmus L, Budak F, Cetinkaya A, Atici EB, Ozkan SA. Development of highly selective and sensitive molecularly imprinted polymer-based electrochemical sensors for tolvaptan assay in tablets and serum. Analytical Methods, 2023; 15(40): 5316-22.
12. Beaudoin JJ, Brock WJ, Watkins PB, Brouwer KL. Quantitative systems toxicology modeling predicts that reduced biliary efflux contributes to tolvaptan hepatotoxicity. Clinical Pharmacology & Therapeutics, 2021 Feb; 109(2): 433-42.
13. Hoshikawa K, Naito T, Saotome M, Maekawa Y, Kawakami J. Validated liquid chromatography coupled to tandem mass spectrometry method for simultaneous quantitation of tolvaptan and its five major metabolites in human plasma. Annals of Clinical Biochemistry, 2019 May; 56(3): 387-96.
14. Murugan S, Kumar NP, Kumar CK, Sundhar VS, Harika S, 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): 17-9.
15. Qazi SS, Khan GJ. Bioanalytical method development and Validation of tolvaptan. Indian J. Applied & Pure Bio, 2025; 40(2): 1155-61.
16. Liu H, Liu T, Liu M, Zheng X, Zhong W, Zhao Q, Jiang J, Hu P, Li Y. Clinical trial on

the pharmacokinetics, pharmacodynamics and safety of tolvaptan in healthy Chinese males: an open-label, single and multiple dosage, parallel group study. Frontiers in Pharmacology, 2025 Nov 21; 16: 1713702.

17. ICH, Q2A text on validation of analytical procedures, 1994.
18. ICH, Q2B validation of analytical methodology, 1996.
19. ICH, Q2 (R1) validation of analytical procedures: text and methodology, 2005.