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Development and Validation of UV Spectrophotometric Method for the Estimation of Tolvaptan in Bulk and Tablet Dosage Form

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ABSTRACT

A simple, rapid, accurate and precise assay procedure based on Spectrophotometric method has been developed for the estimation of Tolvaptan in Pharmaceutical formulation. The method was based on estimation of Tolvaptan at absorption maxiam about 274 nm using methanol as adiluent. The proposed method was linear over the range of 2-20 µg/mL with correlation co-efficient (r^2) of 0.998 and mean recovery for tolvaptan was 99.61 %. The intermediate data obtained under different experimental setup, the calculated value of co-efficient of variance (CV, %) was found to be within a limit. The proposed method can be successfully applied for the analysis of Tablet formulation.

KEYWORDS

Spectrophotometry, Tolvaptan, Validation.

INTRODUCTION

Tolvaptan is a vasopressin antagonist that blocks the binding of arginine vasopressin (AVP) at the V2 receptors of the distal portions of the nephron[1]. Chemically (±)-4'-[(7-chloro-2, 3, 4, 5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-*o* tolu-*m*toluidide[2]. Chemical structure of Tolvaptan is shown in Figure 1. It is not official in any pharmacopoeia, few liquid chromatography procedures have been reported for the determination of Tolvaptan[3-5], but no simple UV/Vis. Spectrophotometric method available for the estimation of Tolvaptan from its formulations. The author have developed a simple UV Spectrophotometry which would serve as a rapid and reliable method for the determination of Tolvaptan in Bulk and pharmaceutical dosage forms.

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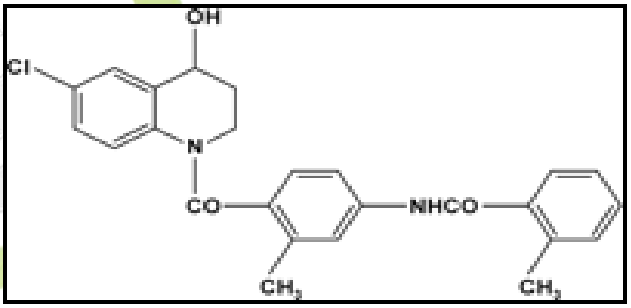

Figure 1: Chemical structure of Tolvaptan

MATERIAL AND METHOD

Instruments and Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.2 nm and pair of 1 cm matched quartz cells, Analytical Balance (CP224S, Sartorius, Germany), Ultrasonic Cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study, and Water Purification System

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(Millipore Bioscience Division Pvt. Ltd, India) was used during study.

Chemicals and Reagents

Kindly gifted reference standards of Tolvaptan (Zydus cadila Healthcare Ltd, Moraiya, Ahmedabad, India), was used without further purification. Tablet preparations containing 10 mg of Tolvaptan was prepared in laboratory. Methanol (A.R. grade; S. D. Fine Chemical Ltd.), Triple Distilled water (Millipore Distilation Unit) prepared in laboratory, and Whatman filter paper no. 41 (Whatman International Ltd., England) was used for the study.

Preparation of Solutions and Tablet Formulation

Standard Stock Preparation for Tolvaptan (100 µg/mL)

Accurately weighed 10 mg Tolvaptan reference standard was transferred in to 100 mL volumetric flask and dissolved in 50 mL of methanol. The solution was sonicated for about 5 minutes with intermittent shaking and diluted to volume with methanol and mixed.

Preparation of Tolvaptan Working Standard Solutions

For calibration curves series of working standard solutions were prepared by transferring varying aliquot of standard stock solution (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 1.6, 1.8 and 2.0 mL) of Tolvaptan to 10 different 10 mL volumetric flask and volume was made up to mark with methanol and mixed.

Preparation of (tablet) Formulation Containing Tolvaptan (10 mg)

Tablets of Tolvaptan were prepared by direct compression technique having average weight about 100 mg. Tolvaptan (API), Cross-carmellose sodium (used as disintegrant), Lactose DCL 11 and MCC PH 102 (used as direct compressible diluent) was shifted through 25# and mixed in a poly bag. Mg stearate (used as lubricant) passed through 60# and added to above blend and mixed. Aerosil (used as glident) was added and mixed. Blend is compressed into tablet using desired punch and dies on single rotary compression press to get desired tablet weight.

Sample Preparation

Twenty tablets were weighed to obtain the average weight. An amount of powdered tablets equivalent to 10 mg of Tolvaptan was transferred in to a 100 mL volumetric flask, and 50 mL methanol was added. The flask was sonicated for 10 minutes and the solution was then filtered through Whatman filter paper No. 41. The residue was washed thoroughly with methanol, filtrate and washings were combined and the volume was made up to 100 mL with methanol. From the above solution 1 mL was further diluted to 10 mL with methanol to achieve final solution having theoretical concentration of Tolvaptan about 10 µg/mL.

RESULTS AND DISCUSSION

Method Development and Selection of Wavelength

For the selection of analytical wavelength, working standard solution of Tolvaptan (10 µg/mL) was scanned in the spectrum mode from 400 nm to 200 nm. The absorbance maximum (λ_{max}) was found to be 274 nm. The UV spectrum for Tolvaptan is depicted in Figure 2.

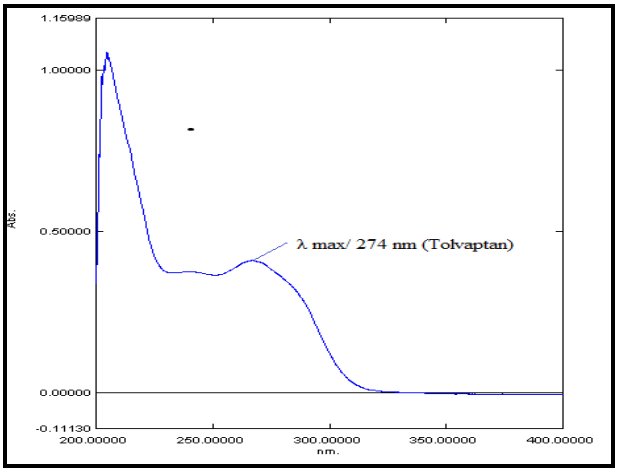

Figure 2: UV Spectra of Tolvaptan in Methanol

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Method Validation[6]

Linearity and Range

The absorbance of working standard solutions of Tolvaptan was measured. The Mean absorbance at each concentration level was calculated and a graph of mean absorbance versus concentration was plotted. The method was linear over the range of 2-20 µg/mL for Tolvaptan. The calibration curve is depicted in Figure 3. The correlation co-efficient, Y intercept, slope of regression line was calculated and recorded in Table 1.

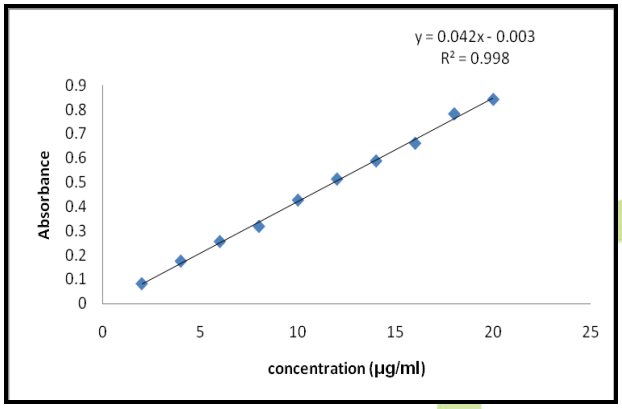

Figure 3: Linearity curve of Tolvaptan

Table 1: Linearity data of Tolvaptan

Sr.no	Conc. of Tolvaptan (µg/mL)	Abs. of Tolvaptan 274 nm (λ_{max})
1	2	0.08129
2	4	0.17564
3	6	0.25962
4	8	0.32214
5	10	0.42501
6	12	0.51225
7	14	0.58703
8	16	0.66004
9	18	0.7821
10	20	0.84162
Correlation coefficient		0.998
Slope of regression line		0.042
Y-intercept		0.003

Accuracy

The accuracy of the method was determined by calculating % recovery of Tolvaptan by standard addition method. Known volumes of standard solutions of Tolvaptan were added at 50, 100 and 150 % levels to preanalyzed sample solutions of 8 µg/mL of Tolvaptan. At each level, three determinations were performed. Result of recovery study is shown in Table 2. The % recovery was found to be varying from 98.83 ± 0.4060 to 101 ± 0.6148 indicate that proposed method was accurate.

Method Precision (% Repeatability)

The precision of the method was checked by repeated measurement of the absorbance values of standard solutions (n = 6) of Tolvaptan (10 µg/mL) without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). The RSD value for Tolvaptan is shown in Table 3. Relative standard deviation was less than 2 %, which indicates that the proposed method was repeatable.

Table 3: Repeatability Data for Tolvaptan by Proposed Method

Concentration (10 µg/mL)	Tolvaptan 274 nm
1	0.4224
2	0.41907
3	0.42501
4	0.42691
5	0.42712
6	0.42612
Mean	0.424438
S.D.	0.003144
RSD	0.7407

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Table 2: Recovery Data for the Tolvaptan (n=6)

Drug	Amount of sample (µg/mL)	Amount of standard added (µg/mL)	Total amount recovered (µg/mL)*	Amount of standard recovered (µg/mL)	% Recovery	RSD
Tolvaptan	8	-	7.98	-	-	0.421
		4	12.02	4.04	101	0.6148
		8	15.9	7.92	99	0.4915
		12	19.76	11.786	98.83	0.4060

n= Number of determinations.

Table 4: Intra-day Precision Data for Tolvaptan

Conc. (µg/mL) Tolvaptan	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean absorbance ± RSD (n=3)
4	0.17258	0.17542	0.17454	0.17418 ± 0.834667
8	0.31978	0.31638	0.31814	0.3181 ± 0.534531
12	0.50623	0.51225	0.51424	0.51090 ± 0.816301

n= Number of determinations.

Table 5: Inter-day Precision Data for Tolvaptan

Conc. (µg/mL) Tolvaptan	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean absorbance ± RSD (n=3)
4	0.17124	0.17654	0.17454	0.174107 ± 1.5372
8	0.31616	0.32214	0.31814	0.318813 ± 0.955522
12	0.50623	0.5172	0.51424	0.512557 ± 1.1072

n= Number of determinations.

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Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of Tolvaptan (4, 8 and 12 µg/mL). The results were reported in terms of relative standard deviation (RSD). The RSD values for Tolvaptan are recorded in Tables 4 & 5. Relative standard deviation was less than 2 %, which indicates that the proposed method was repeatable.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = the standard deviation of the response, S = slope of the calibration curve.

LOD and LOQ were calculated by equation using signal to noise ratio. As per ICH guideline, which were found to be 0.7 µg/mL and 2.1 µg/mL, respectively.

Assay of the Pharmaceutical Formulation

Absorbance values of the sample solutions were measured at 274 nm. The amount of Tolvaptan present in sample solution was determined by fitting the absorbance response into the regression equation of Tolvaptan in the method. Results are shown in table 6. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine estimation of Tolvaptan in pharmaceutical dosage forms.

CONCLUSION

Based on the results obtained from the analysis of the described method, it can be concluded that the method has linear response in the range of 2-20 µg/mL for Tolvaptan. 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.

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Table 6: Analysis of Marketed Formulation of Tolvaptan by Proposed Method

Sample No.	Label Claim	Amount Found	% Label Claim
	Tolvaptan (mg/tab)	Tolvaptan (mg/tab)	Tolvaptan (mg/tab)
1	10	9.88	98.8
2	10	9.72	97.2
3	10	10.03	100.3
4	10	10.20	102
5	10	10.08	100.8
6	10	9.92	99.2
Mean		9.9716	99.71
S.D.		0.1683	1.683

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