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An Eco-friendly RP-HPLC and UV-Method Development and Validation for an Estimation of Tolvaptan in Bulk and Tablet Dosage form Followed by Forced Degradation Studies

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To develop and validate a new, simple, rapid, precise, and accurate An Eco-friendly RP-HPLC and UV-Method Development and Validation for an estimation of Tolvaptan in Bulk and Tablet dosage form followed by Forced Degradation Studies
Place and Duration of the Study: The present work has been carried out at Ali-Allana College of Pharmacy, Akkalkuwa between November-2020 to April-2021.
Methodology: The UV-Spectroscopic method was developed for the estimation of tolvaptan in bulk and tablet dosage form. The solvent selected for the tolvaptan UV analysis was 4% aq. SLS solution, the solution of 10µg/ml was scanned in UV region from 200-400 nm and the λmax value was determined. The RP-HPLC method was developed on Sunsil C18 150 mm x 4.6mm x 5µ column using acetonitrile: water [45:55] as mobile phase at flow rate 1.0 ml/min and UV detection at 266 nm.
Results: The maximum absorbance was observed at 266 nm. The wavelength 266 nm was selected for further analysis of tolvaptan. The calibration curve was determined using drug

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concentrations ranging from 20-100 µgm/ml. The system suitability was performed by injecting a standard solution containing 200µg/ml of tolvaptan in six replicates. For two of them, the peak asymmetric were <1.5 and the theoretical plate number was >2000, and the %RSD of tolvaptan was less than 2.

Conclusion: From the above results, it was concluded that the developed UV and RP-HPLC methods are precise and accurate and can be applied for the quantitative estimation of tolvaptan from bulk and tablet dosage forms. The method can be used for routine testing of tolvaptan by the pharmaceutical industry. Validation of the developed method was done as per International Conference on Harmonization (ICH) Q2R1 guidelines.

Keywords: Reverse phase high-performance liquid chromatography; UV-visible spectroscopy, method development; validation, tolvaptan; international conference on harmonization (ICH) Q2R1.

ABBREVIATIONS

RP-HPLC	:	*Reverse Phase High-Performance Liquid Chromatography*
ICH	:	*International Conference on Harmonization*
LOD	:	*Limit of Detection*
LOQ	:	*Limit of Quantification*
AVP	:	*Arginine Vasopressin*
CHF	:	*Congestive Heart Failure*
CYP3A4	:	*Human Cytochrome P450 3A4*

1. INTRODUCTION

Tolvaptan is a selective arginine vasopressin (AVP) V2 receptor blocker used to induce free water diuresis in the treatment of euvolemic or hypervolemic hyponatremia. Currently, the orally active medication is in the final stages before approval by the FDA for outpatient therapy. It appears to be safe and effective at promoting aquaresis and raising serum sodium levels in both short- and long-term studies [1-2]. Tolvaptan is also effective for the treatment of congestive heart failure (CHF) exacerbation, but whether there are long-standing beneficial effects on CHF is still controversial. Prolonged use of tolvaptan leads to increased endogenous levels of AVP and perhaps over-stimulation of V1A receptors. Theoretically, this activation could lead to increased afterload and cardiac myocyte fibrosis, causing the progression of CHF. However, after 52 weeks of tolvaptan therapy, there was no worsening of left ventricular dilatation. In addition, tolvaptan is metabolized by the CYP3A4 (Human Cytochrome P450 3A4) system; thus physicians should be aware of the potential for increased interactions with other medications [3-4]. Tolvaptan is a breakthrough in the therapy of hyponatremia as it directly combats elevated AVP levels associated with the syndrome of inappropriate secretion of antidiuretic hormone, congestive heart failure, and cirrhosis of the liver [5].

Literature survey reveals few UV spectrophotometers, HPLC, UPLC, LC/MS-MS methods for compound estimation in bulk and pharmaceutical dosage form [6,7-13]. Based on the literature review, stability-indicating RP-HPLC and UV-Visible method for the estimation of tolvaptan was not found. Hence, it was felt that there is a need for a new analytical method.

The present work aimed to develop a simple, accurate, precise, rapid, specific, sensitive, and selective stability-indicating RP-HPLC and UV-Visible method for the estimation of tolvaptan in bulk and tablet dosage form.

2. MATERIALS AND METHODS

2.1 Instruments

Shimadzu HPLC system (LC-20AD Multi-solvent delivery system, SPD-20A, UV-Visible Detector, LC solution software). UV-Visible Spectrophotometer (Shimadzu- 1800 double beam, with UV Probe 2.33). Labman sonicator was used for sonication of the sample solution. Thermo scientific pH meter was used to measure pH. A vacuum pump filter was used for the filtration of mobile phase solvents.

2.2 Chemicals

The pure drug sample of tolvaptan was procured from Hetero Drug Limited and the tablet dosage form was purchased from Roop Agencies, Pharmaceutical Distribution. SLS (Sodium Lauryl Sulphate) was purchased from Research Lab fine Chem Industry, Mumbai. Acetonitrile and HPLC grade water was procured from Termosil Fine Chem Industry, Ankleshwar, India.

3. CHROMATOGRAPHIC CONDITIONS

The isocratic mobile phase consisted of Acetonitrile: Water [45:55], flowing through the column at a constant flow rate of 1.0 ml/min. Sunsil C18 column (150 mm x 4.6mm x 5µm) was used as the stationary phase. 266 nm was selected as the detection wavelength for the UV-Visible detector.

4. DEVELOPMENT OF UV SPECTROSCOPIC METHOD FOR THE QUANTIFICATION OF TOLVAPTAN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

4.1 Preparation of Standard Stock Solution

An appropriate weight of 100 mg of tolvaptan was transferred into 100 ml of the volumetric flask containing 50 ml of SLS (4% w/v) solution, sonicated for 15 minutes and volume made up to 100 ml with the same solvent to an obtained concentration of 1000 µg/ml.

A fixed volume of 10 ml of the above solution (1000 µgm/ml) was transferred into a 100 ml volumetric flask and the volume was made up to 100 ml with the same solvent (SLS 4% W/V) to obtain a concentration of 100 µg/ml. The working standard was prepared by dilution of the standard stock solution [14-15].

4.2 Determination of λmax

A fixed volume of 1.0 ml of tolvaptan from the standard stock solution was transferred to a 10 ml volumetric flask and diluted up to the mark with SLS (4% w/v) to obtain a concentration of 10 µgm/ml. The resultant solution was scanned in a UV spectrophotometer in the range (400-200 nm) in a 1.0 cm cell against solvent blank [16].

4.3 Validation of the Developed Method

The developed method was validated as per the ICH guidelines in terms of linearity, precision, accuracy, repeatability, and stability studies.

a) Linearity

Linearity was assessed by measuring several analyte concentrations varying quantities of standard stock solution was diluted with the SLS (4% w/v) solution to give a 5, 10, 15, 20, and 25 µg/ml concentration. The calibration curve was obtained by plotting the absorbance against concentration (µg/ml) [17].

b) Precision

Precision studies were carried out to ascertain the reproducibility of the developed method. An inter-day precision study was carried out by preparing a drug solution of three different concentrations (10, 15, and 20 µgm/ml of tolvaptan) and analyzing it at three different times in a day. The intraday precision study was carried out by preparing a drug solution of three different concentrations (10, 15, and 20 µg/ml of tolvaptan) and analyzing it on three different days [18].

c) Accuracy

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in a pre-analyzed sample solution of 4 µg/ml. To pre-analyzed sample solution, a known amount of working standard solution of tolvaptan (0.33, 0.42, and 0.48 ml of 100 µg/ml) was added in 10 ml volumetric flask and made up to the mark with diluent which was at different level i.e. 80%, 100%, and 120%. The solutions were analyzed by the proposed method. The mean % recovery from peak areas obtained was calculated [19].

d) Repeatability

Repeatability was determined by preparing six replicates of 15 µgm/ml of tolvaptan and the absorbance was measured at 266 nm [20].

e) Limit of Detection (LOD) and Limit of Quantification (LOQ)

1) LOD

The LOD was estimated from the set of five calibration curves used to determine method linearity. The calibration curve was repeated for 6 times and the SD of the intercept was calculated then LOD was calculated as follow:

LOD= (3.3*SD)/slope.
Where,
SD= the standard deviation of the y-intercept of 5 calibration curves.
Slope= the mean slope of the 5 calibration curves.

2) LOQ

The LOQ was estimated from the set of five calibration curves used to determine method linearity. The LOQ may be calculated as

LOQ = 10 × (σ/S).

Where,
σ = Standard deviation of the Y- intercepts of the five calibration curves.
S = Mean slope of the five calibration curves [21-23].

4.4 Quantification of Tolvaptan in Formulation

The 10 tablets of tolvaptan (15mg) were accurately weighed and transferred to a dry and clean mortar, then grind into a fine powder, the tablet powder was weighed equivalent to 10 mg and transferred to 100 ml volumetric flask containing 50 ml of 4% SLS solution. The mixture was sonicated for 15 min to dissolve and make volume up to the mark with 4% SLS solution (100 mcg/ml), transfer the 2 ml from this solution to 10 ml volumetric flask and dilute up to the mark with 4% SLS solution which will form solution of 20 µgm/ml concentration [23].

5. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR TOLVAPTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY HPLC

5.1 RP-HPLC Method Development and Optimization

5.1.1 Chromatographic separation

Standard solutions of tolvaptan were injected in a column with a 20µl micro-syringe. The chromatogram was run for appropriate minutes (10 min) with mobile phase acetonitrile: water (45:55). The detection was carried out at wavelength 266 nm. The chromatogram was stopped after separation was achieved completely. Data related to peaks like area, height, retention time, etc. were recorded using software [23-24].

5.1.2 Chromatographic conditions

Column: C18
Mobile Phase: Acetonitrile: water (45:55)
Flow Rate: 1.0 ml/min
Detection Wavelength: 266 nm
Run time: 10 min
Injection volume: 20.0 µL

5.1.3 Optimization of mobile phase

The selected mobile phase i.e. acetonitrile and water have been tried in different proportions to get the most optimized peak.

5.1.4 Preparation of diluent and standard stock solution

The standard stock solution of tolvaptan was prepared by accurately weighing 100 mg of tolvaptan drug and transferred into a 100 ml volumetric flask containing 50 ml of diluent. The above mixture was sonicated to dissolve and the volume made up the mark with diluent to an obtained concentration of 1000 µgm/ml. A fixed volume of 5.0 ml of the above solution [1000 µgm/ml] was transferred into 100ml of volumetric flask and diluted up to the mark with diluent to an obtained concentration of 500µg/ml. The working standard was prepared by dilution of the standard stock solution [24,7,8]

5.2 HPLC Method Validation

a) System Suitability

The system suitability parameters and their limits are tabulated in table 5. The system suitability was performed by injecting a standard solution containing 200µg/ml of tolvaptan in six replicates. For two of them, the peak asymmetric were <1.5 and the theoretical plate number was >2000, and the %RSD of tolvaptan was less than 2. The result indicates that the system suitability parameter was within the acceptable limit [9].

b) Linearity

The linearity for tolvaptan was assessed by analysis of standard solution in the range of 35-175 µgm/ml and 0.7,1.4,2.1,2.8, and 3.5 ml of solutions were pipette out from the Stock solution of tolvaptan (500 µgm/ml) and transferred to 10 ml volumetric flask and makeup with diluent to obtain 35,70,105,140, and 175 µgm/ml. The linearity for tolvaptan was assessed by analysis of standard solution in the range of 35-175 µgm/ml. The correlation coefficient for calibration curve tolvaptan was found to be 0.997 [9].

c) Precision

The precision of the developed method has been checked by studying it on interday and intraday. A standard solution containing (70, 140, 210 µgm/ml) of tolvaptan was analyzed three times on the same day and the different three days and % R.S.D was calculated [10].

d) Accuracy

100 µgm/ml drug solution was taken in three different flask labels A, B and C. Spiked 50%, 100%, and 150% of the standard solution in it and diluted up to 10 ml. The area of each solution peak was measured at 266 nm. The amount of tolvaptan was calculated at each level and % recoveries were computed [10].

e) Robustness

For robustness study, the following parameters have been changed one by one and their effects on system suitability were observed.
i) Change mobile phase composition by ± 1.0 mL of organic solvent.
ii) Change Wavelength ± 1 nm
iii) Change flow rate ±0.1 mL/min.

Change in mobile phase composition: Std. the working solution was injected three times by change in the mobile phase composition by ±1.0 mL of organic solvent (Acetonitrile: water) (44.11v/v and 56.09v/v) of the developed method.

Change in wavelength: Std. the working solution was injected three times by change in the Wavelength by ± 1 nm of a sample (265 nm and 267 nm) of the developed method. Calculate the% RSD of the mean area for change in the method parameter.

Change in flow rate: Std. the working solution was injected three times by change in the flow rate by ± 0.1 mL/min (0.9 mL/min and 1.1mL/min) of the developed method. Calculate the %RSD of mean area for change in method parameter [11].

g) Repeatability

A standard solution containing tolvaptan (35 µg/ml) was injected six times and areas of peaks were measured and % RSD was calculated [12].

5.3 Analysis of Marketed Formulation

a) Preparation of standard solution for assay

60 mg of accurately weighed tolvaptan (API) was transferred in a 100 ml of volumetric flask. Then 60 ml of diluent was added and sonicated to dissolve the drug completely. The contents were diluted with diluent up to the mark. The 5 ml of the above solution was transferred into a 50 ml volumetric flask, diluted to volume with diluent, and mixed [12-13].

Table 1. Linearity of tolvaptan (UV)

Sr. No.	Conc. (µgm/ml)	absorbance	Mean	SD	%RSD
1	5	0.159	0.160	0.001528	0.95
		0.161			
		0.162			
2	10	0.296	0.294	0.002	0.68
		0.294			
		0.292			
3	15	0.409	0.406	0.002517	0.62
		0.407			
		0.404			
4	20	0.539	0.541	0.002517	0.46
		0.541			
		0.544			
5	25	0.652	0.652	0.003	0.46
		0.649			
		0.655			
Correlation coefficient (r^2)		0.999			
Regression Equation		Y = 0.0246x+0.0413			

Table 2. System suitability parameters of the developed method

Sr. No.	System Suitability Parameter	%RSD	
1	Linearity	0.46-0.95%	
2	Accuracy	0.63 to 0.91%	
3	Precision	Interday	Intraday
		0.72-0.87%	0.52-1.71%
4	Repeatability	0.74	
5	LOD	1.2845	
6	LOQ	3.893	

Table 3. The data of tolvaptan assay

The label claimed in mg	The average weight of tablet (mg)	Absorbance at 266nm	The label claimed found in mg	% assay
15 mg	97.6 mg	0.674	15 mg	100%

Table 4. The different ratios of mobile phase tried and remark on obtained peaks

Mobile Phase	Flow Rate (ml/min)	Ratio	Retention Time (min) tolvaptan	Remark
Acetonitrile: water	1.0	30:70	8.087	One peak was observed with irregular shape and high retention time
	1.0	35:65	7.558	Broad peak
	1.0	40:60	7.225	Still, peak shape is not good
	1.0	50:50	6.693	Peak shape was not good
	0.8	45:55	5.845	RT is more and peak shape still not good
	0.8	45:55	5.835	Run time decreased but the efficiency of the peak was low
	0.9	45:55	5.014	Run time decrease but the efficiency of the peak was low
	1.0	45:55	4.798	Satisfactory peak

Table 5. The system suitability results of the developed method

Parameter	Result
Theoretical plates per column	Not less than 2000
Symmetric factor/tailing factor	Not more than 2
Retention time	About 3-5 minutes

Table 6. The linearity data of tolvaptan

Sr. No.	Concentration (µgm/ml)	Area
1	35	1552155
2	70	2966969
3	105	4217426
4	140	5909046
5	175	7373362
Correlation coefficient r^2		0.9979
Regression equation		y = 41670x+28444
%RSD		0.91%-1.71%

b) Preparation of sample solution for assay

The 10 tablets were crushed and accurately weighed and blended into a fine powder. Take accurately weighed and transferred the powder equivalent to 120 mg into a 200 ml volumetric flask. About 120 ml of diluent was added and sonicated for 15 min. Transfer 5 ml of the

solution into 50 ml volumetric flask dilute with diluent and mix. Filter the solution through a 0.45 µ filter [13].

5.4 Degradation Studies of Tolvaptan

a) Acid Hydrolysis

1.0 ml of aliquot was taken from a stock solution in 10 ml of volumetric flask. To this 2.0 ml of 0.1N HCl was added and the solution was allowed to stand for 4 hours and neutralized using 0.1N NaOH. Finally, dilute up to the mark using a diluent to get the final concentration of 10 µgm/ml of tolvaptan.

b) Alkali Hydrolysis

1.0 ml of stock solution was taken in 10 ml of volumetric flask. To this 2 ml of 0.1 N NaOH was added and then solutions were allowed to stand for 4 hours and neutralizing using 0.1N HCl. Finally diluted up to the mark using a diluent to get a final concentration of 10 µg/ml of tolvaptan.

c) Oxidative Hydrolysis

1.0 ml of an aliquot from stock solution was taken in 10 ml of the volumetric flask, to this 2.0 ml of 30% hydrogen peroxide was added and then the solution is allowed to stand for 4 hours. Finally, the contents were diluted up to the mark using a diluent to get a final concentration of 10 µg/ml of tolvaptan.

d) Thermal Hydrolysis

100 mg sample was weighed and kept in an oven at 60-70°C for 24 hours. From this 10 mg sample was weighed and transferred in 10 ml of volumetric flask. The volume was made up to the mark with diluent. Then 1ml of the above solution was taken and transferred in 10ml of volumetric flask and the volume made up to the mark with diluents. This solution was injected into the system.

e) Photolytic Hydrolysis

100 mg of sample was weighed and kept in sunlight for 4 hours. From this 10 mg of sample was weighed and transferred in a volumetric flask. Make up the volume up to the mark with diluents. Then 1 ml of the above solution was taken and transferred in 10 ml of volumetric flask. Make up the volume with diluents.

f) UV Degradation Study

100 mg sample was weighed and kept in UV chamber at 200-400nm for 24 hours and 48 hours from this 10 mg of sample was weighed and transferred in 10 ml volumetric flask. Make up the volume up to the mark with diluents. Then take 1 ml of the above solution and transfer it into 10 ml of volumetric flask. Make up the volume with diluents. This solution was injected into the system [12-13].

6. RESULTS AND DISCUSSION

6.1 Development of UV Spectroscopic Method for the Quantification of Tolvaptan in Bulk and Pharmaceutical Dosage Forms

The UV spectroscopic method was developed for the estimation of tolvaptan in the form of bulk and tablet doses. The solvent selected for the UV analysis of tolvaptan was a 4% aqueous solution. SLS solution, the 10 µg / ml solution was scanned in the UV region of 200-400 nm and the λmax value was determined. The maximum absorbance was observed at 266 nm, the UV plot is shown in Fig. 1. The wavelength of 266 nm was selected for further analysis of tolvaptan. This developed UV spectroscopic method has been validated according to ICH guidelines in terms of linearity, precision, accuracy, repeatability, and stability studies [1-2].

Linearity was evaluated by measuring at a concentration of various analytes, variable quantities of standard stock solution were diluted with SLS solution (4% w / v) to give a concentration of 5, 10, 15, 20, and 25 µgm/ml. The calibration curve was obtained by plotting absorbance as a function of concentration (µgm/ml) in Fig. 2. From this, it was observed that absorbance increases with increasing concentration. The absorbance of each concentration was estimated in triplicate and the% RSD was calculated. The% RSD was found to be between 0.46-0.95%, which is satisfactory and permissible under ICH guidelines. Precision studies were performed to verify the reproducibility of the developed method. The between-day precision study was performed by preparing a drug solution at three different concentrations (10, 15, and 20 µgm / ml tolvaptan) and testing it at three different times in one day. An intraday precision study was carried out analyzing it over three different days. For the intraday, it was found that the% RSD was

between 0.52-1.71%, while for the interday it was found between 0.72-0.87%. % RSD accuracy was considered satisfactory and permissible according to ICH guidelines. Precision was determined by performing recovery studies by adding different concentrations of pure drug to a previously tested 4 µg / mL standard solution. The% concentration of 80, 100, and 120 analyzed with the developed method and the% recovery were studied in terms of% RSD. The% RSD value was found to be between 0.63 and 0.91%, which is satisfactory. Repeatability was determined by preparing six replicates of 15 µg/mL tolvaptan and absorbance was measured at 266 nm. The mean% RSD was found to be 0.74, indicating that the results are reproducible and accurate. The LOD and LOQ were determined and found to be 1.2845 and 3.893, respectively given in Tables 2 [3-5].

This developed UV spectroscopic method has been successfully applied for the quantitative estimation of tolvaptan in the commercial tablet formulation. From the tablet formulation using a 4% SLS solution, a tolvaptan concentration of 20 µgm / ml was prepared and subjected to UV analysis. The UV spectra of the commercialized formulation are illustrated in Fig. 3. The% of the test was found to be 100% given in Table 3 and the estimated quantity was the same as indicated on the label. From the above results, it can be concluded that this developed UV spectroscopic method was accurate and precise for the quantitative estimation of tolvaptan in the bulk and tablet formulation.

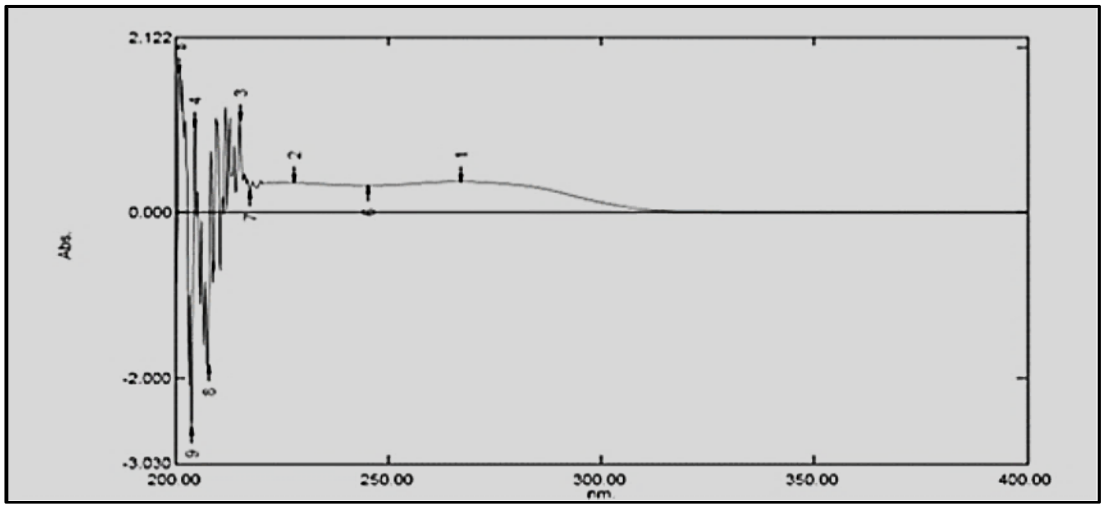

Fig. 1. UV spectrum of standard tolvaptan for the selection of wavelength (λmax at 266 nm)

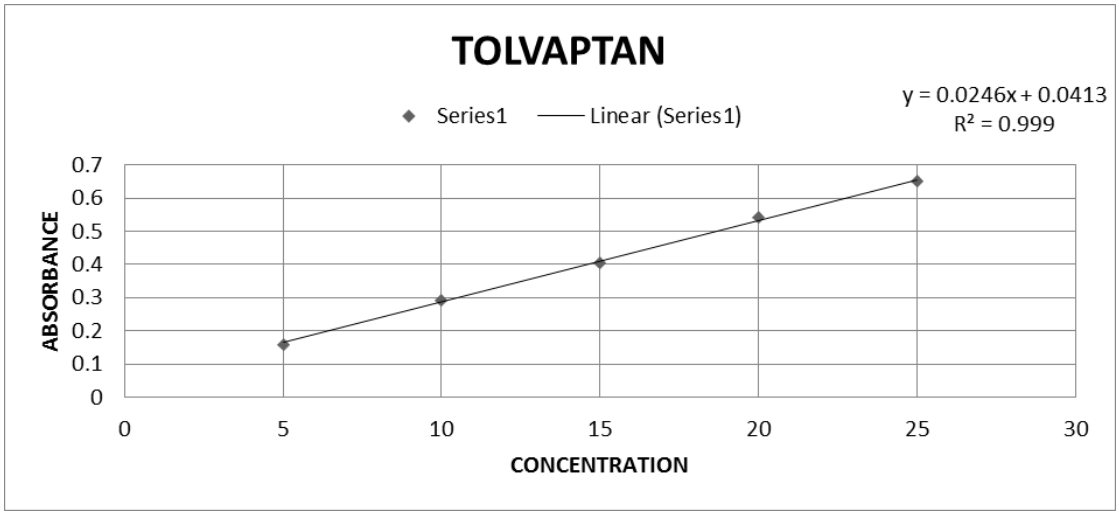

Fig. 2. The calibration curve of tolvaptan (UV)

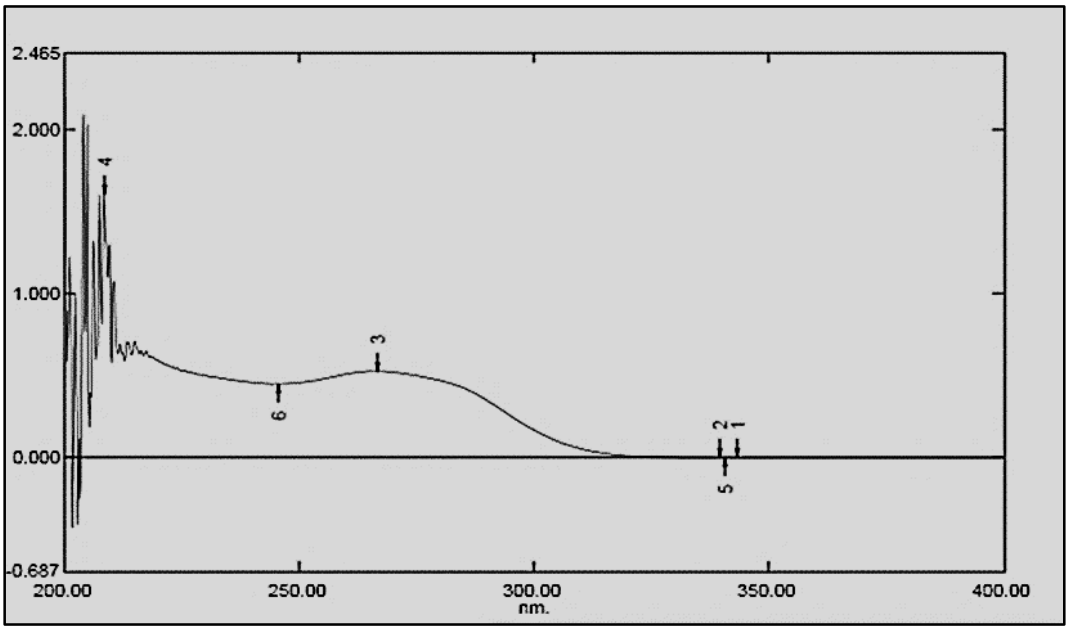

Fig. 3. The UV Spectra of tolvaptan assay (Marketed Formulation)

6.2 Analytical Method Development and Validation for Tolvaptan in Bulk and Pharmaceutical Dosage form by HPLC

The RP-HPLC method for quantitative estimation of bulk and tablet tolvaptan was developed and validated according to ICH guidelines. The method was developed by selecting a detection wavelength of 266 nm. The selected mobile phase, namely acetonitrile, and water, was tested in different proportions to obtain the most optimized peak. The different proportions were shown in Table 4. The most optimized peak was obtained with the mobile phase acetonitrile: water (45:55). The most optimized peak of tolvaptan is illustrated in Fig. 4. The optimized chromatographic conditions were: C18 column with a run time of 10 min and a retention time of 4.7 min, the most resolved tolvaptan peak was obtained with a flow of 1 ml/min [18-23].

This developed RP-HPLC method has been validated according to ICH guidelines in terms of suitability, linearity, precision, accuracy, repeatability, and system stability studies. System suitability was achieved by injecting a standard solution containing 200 µg / ml of tolvaptan in six repetitions. For two of them, the asymmetric peak was <1.5 and the theoretical number of plates was> 2000 and the% RSD of tolvaptan was less than 2. The result indicates that the suitability parameter of the system was within the acceptable limit. The linearity of tolvaptan was evaluated by analysis of the standard solution in the range of 35-175 µgm / ml. The correlation coefficient for the tolvaptan calibration curve was found to be 0.997 as shown in Fig 5. The% RSD value was found to be between 0.91 and 1.71. The standard solution containing (70, 140, 210 µg / ml) of tolvaptan was analyzed three times on the same day and the three different days and the% R.S.D. For intraday, the% RSD was found to be between 0.66 and 1.92% and for interday, it was found between 0.68 and 1.96%, which is satisfactory and admissible according to the guidelines of the 'ICH. LOD and LOQ were found at 4,693 and 14,221 respectively. Precision studies were performed by calculating the% recovery of the drug solution with a% concentration of 50, 100, and 150. The mean% recovery was between 99.90 and 100.13%. It is permitted by the ICH guidelines and is considered satisfactory. The robustness studies were carried out by modifying some parameters, such as the composition of the mobile phase, altering the wavelength, and varying the flow rate. We find very low RSD% values when we change each parameter of the developed method. The repeatability of the method was determined by analyzing the same concentration 6 times and the% RSD was found to be 1.03, which is permissible according to the ICH guidelines. The stability of the analytical solution was verified by initially analyzing the standard and filtered sample solution and also at different time intervals, as indicated below, by

storage in the sample compartment of the HPLC instrument under ambient conditions [23-6].

This developed RP-HPLC method has been successfully applied for the quantitative estimation of tolvaptan in the commercial tablet formulation. The 10 tablets were crushed and precisely weighed and mixed into a fine powder. Take the exact weight and transfer the powder equivalent to 120 mg into a 200 ml volumetric flask. Approximately 120 ml of diluent was added and sonicated for 15 min. Transfer 5 ml of the solution to a 50 ml volumetric flask diluted with diluent and mix. Filter the solution through a 0.45 µ filter. The most optimized chromatogram obtained from the tablet formulation is shown in Fig. 6. The% of the study was found to be 101.87%, as allowed by the ICH guidelines [24].

Degradation studies in tolvaptan solution were conducted at different conditions using a developed method as shown in Fig. 7 to Fig. 13. The% degradation was tabulated in Table 13. The highest degradation was observed in acid hydrolysis of 18.24% and the lowest was observed due to sunlight, ie 0.27. In the HPLC solubility study of tolvaptan, the mean solubility was estimated to be 80.66% using a 4% SLS solution.

From the above results, it was concluded that the developed method is precise and accurate and can be applied for the quantitative estimation of tolvaptan from bulk and tablet dosage forms. The method can be used for routine testing of tolvaptan by the pharmaceutical industries [7-13].

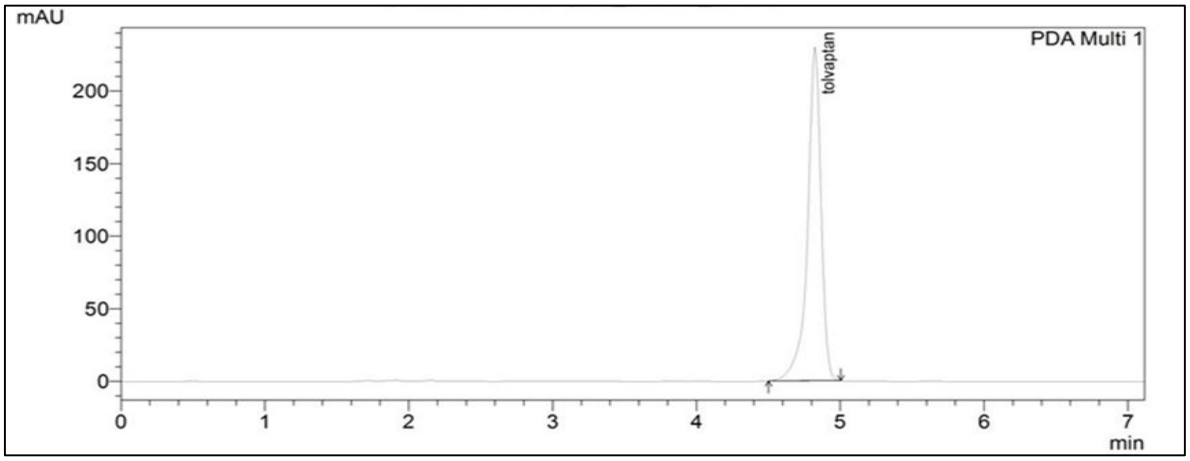

Fig. 4. The best-optimized chromatogram of tolvaptan in bulk

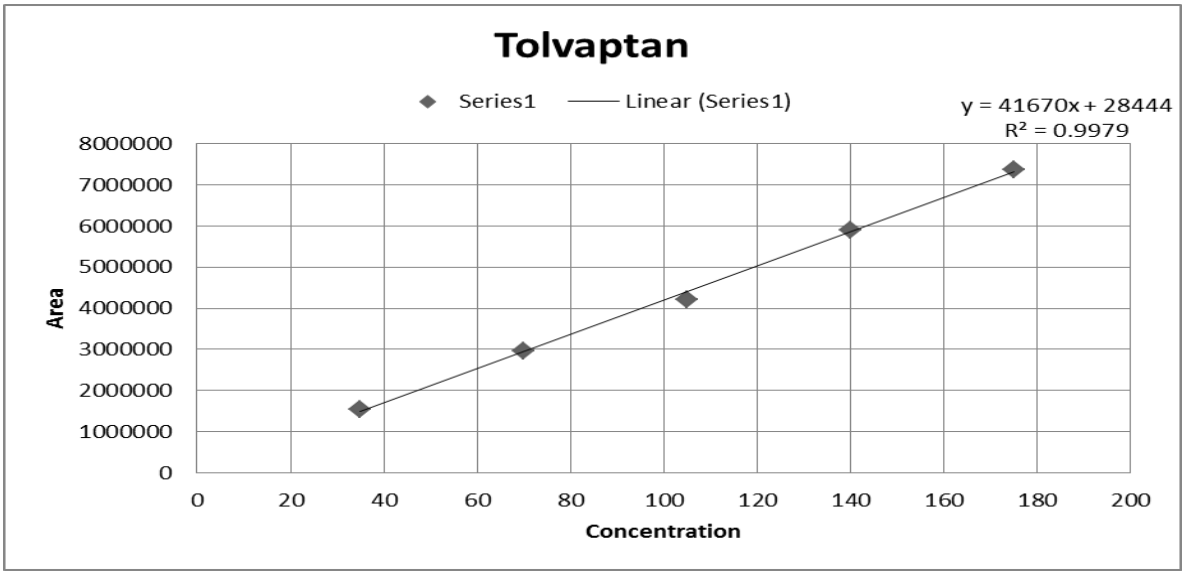

Fig. 5. The calibration curve of tolvaptan [HPLC]

Table 7. The precision data of tolvaptan for HPLC method validation

Sr. No.	Conc. (µgm/ml)	Area	II	III	Mean	SD	%RSD
Intraday							
1	70	3105499	3005499	3105500	3005499.00	57735.32	1.92
2	140	5788060	5688060	5788062	5754727.33	57735.60	1.00
3	210	8757192	8657192	8757195	8723859.67	57735.89	0.66
Interday							
1	70	2949671	2949675	2849671	2949675.00	57736.18	1.96
2	140	5778196	5678196	5778199	5744863.67	57735.89	1.01
3	210	8523602	8423602	8523605	8490269.67	57735.89	0.68

Table 8. The accuracy data of tolvaptan of HPLC method validation

% concentration	Sample amount (µgm/ml)	Amount added (µgm/ml)	Amount recovered (µgm/ml)	% recovery	% Recovery means	S.D.	% RSD
50%	12.5	7.5	7.56	100.8	99.90	0.87	0.871
	12.5	7.5	7.49	99.86		0	
	12.5	7.5	7.43	99.06			
100%	20	15	14.92	99.46	100.13	1.64	1.63
	20	15	15.3	102			
	20	15	14.84	98.93			
150%	27.5	22.5	22.3	99.11	100.5	1..31	1.31
	27.5	22.5	22.7	100.66			
	27.5	22.5	22.89	101.73			

Table 9. The robustness data of tolvaptan

Condition	Peak area mean	SD	% RSD
Change in ratio of mobile phase ± 1ml	5962632	24880.24	0.42
	6058536	73565.62	1.21
Change in wavelength ± 1nm	5975503	78830.27	1.31
	6065253	67944.48	1.12
Change in flow rate ± 1ml	5920746	58860.81	0.99
	6125365	46099.98	0.75

Table 10. The repeatability data of tolvaptan

Sr. No.	Conc. (µgm/ml)	Area	Mean	SD	%RSD
1	35	1529816	1561062	16148.4	1.03445
2	35	1530789			
3	35	1529865			
4	35	1528845			
5	35	1530852			
6	35	1529856			

Table 11. The LOD and LOQ data of tolvaptan

Parameters	Result
SD of intercept	0.0023124
Slope	0.0246
LOD(µg/ml)	0.3102
LOQ(µg/ml)	0.94

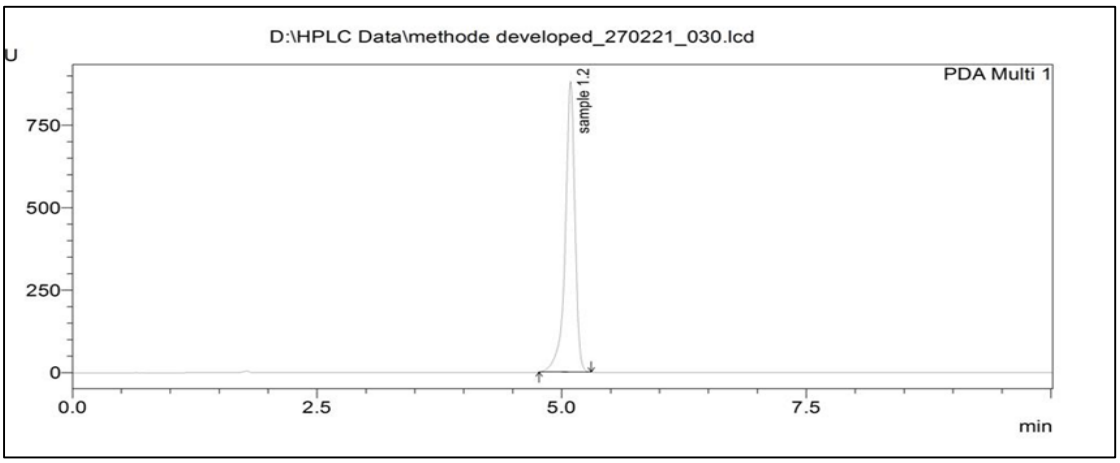

Fig. 6. The optimized chromatogram of tolvaptan formulation

Table 12. The assay data of tolvaptan

Sr. No.	Label Claim (mg)	Area of Standard	Area of Sample	Result (mg)	% assay	Average % assay
1	15	5947896	6034632	15.199	101.33%	
2	15	5971076	6062383	15.216	101.44%	101.87%
3	15	6034632	6214396	15.426	102.84%	

Table 13. The data of degradation study of tolvaptan

Degradation	Interval (hours)	Peak area	% Non-degradation	% Degraded
Acid	0-4	2870982	81.76	18.24
Base	0-4	2916657	83.07	16.93
Hydrogen peroxide	0-4	3018943	85.98	14.02
Thermal	0-48	3278118	93.36	6.64
UV light	0-24	3409482	97.10	2.9
	0-48	3134416	89.27	10.73
Sunlight	0-4	3501789	99.73	0.27

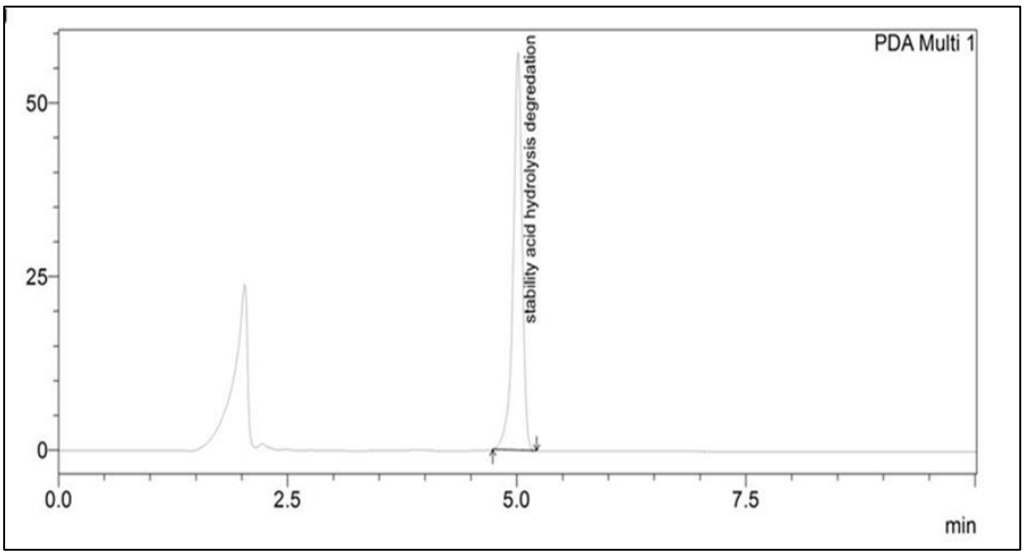

Fig. 7. The chromatogram of tolvaptan obtained in the acid stability study

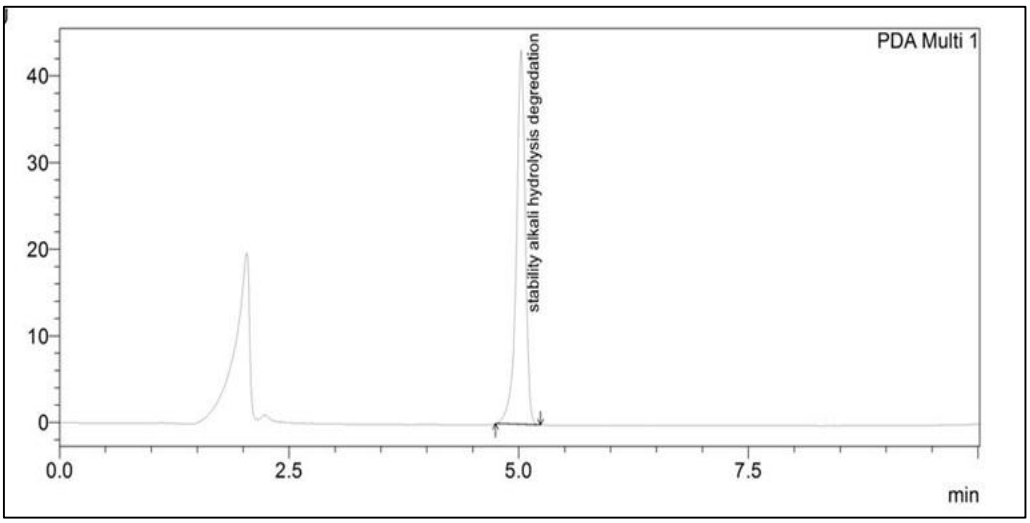

Fig. 8. The chromatogram of tolvaptan obtained in alkali hydrolysis study

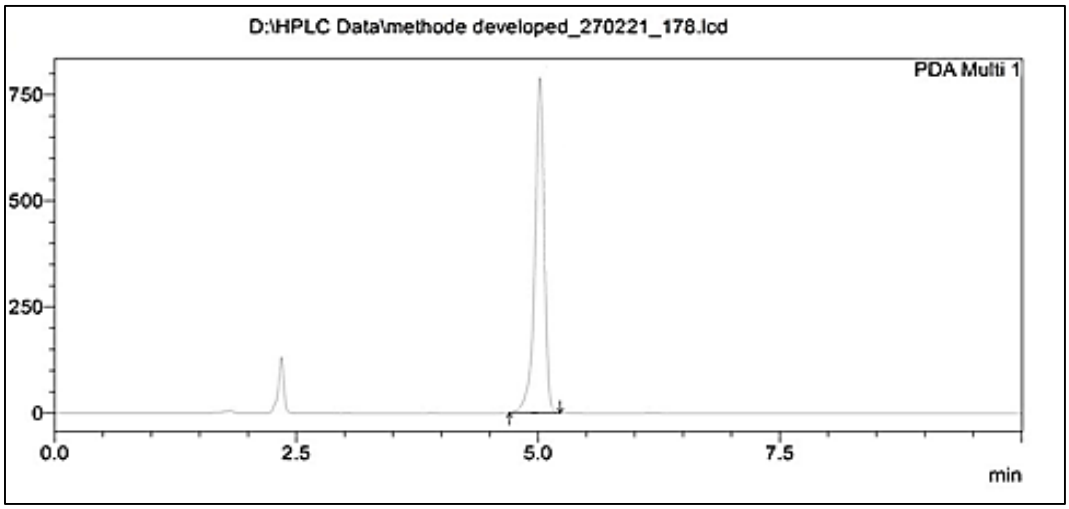

Fig. 9. The chromatogram of tolvaptan obtained in oxidative hydrolysis study

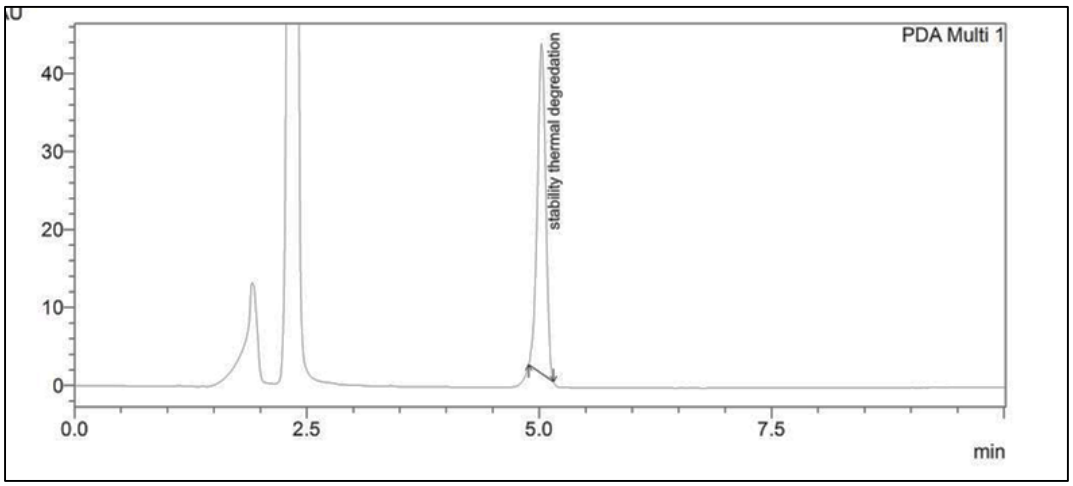

Fig. 10. The chromatogram of tolvaptan obtained in thermal hydrolysis study

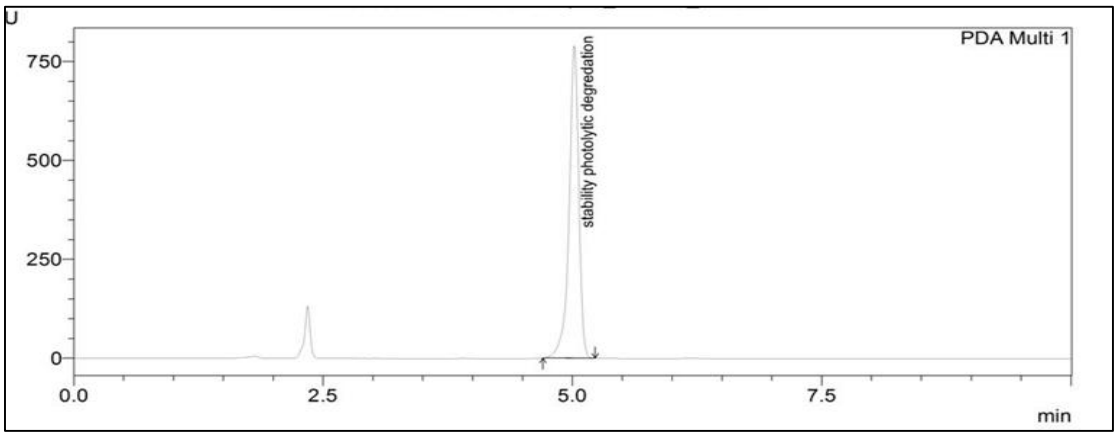

Fig. 11. The chromatogram of tolvaptan obtained in the photolytic degradation study

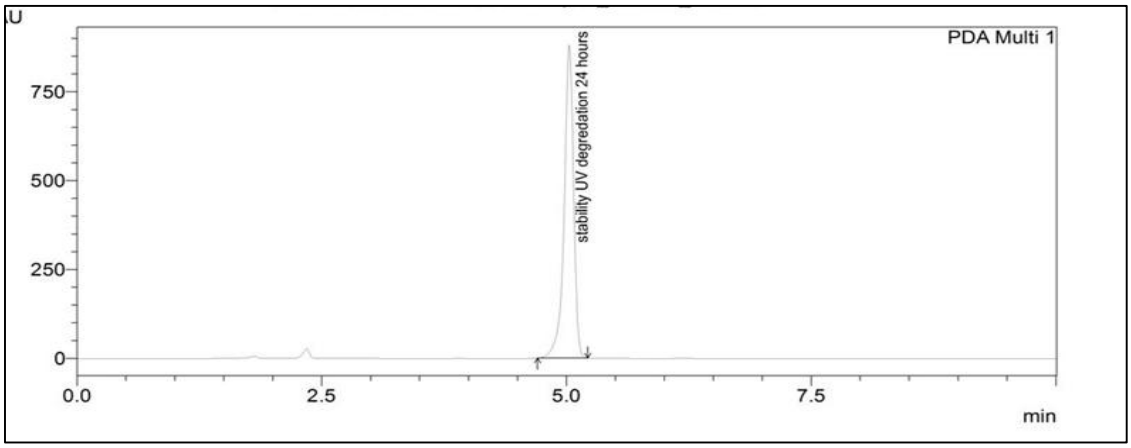

Fig. 12. The chromatogram of tolvaptan in UV degradation study after 24 hours

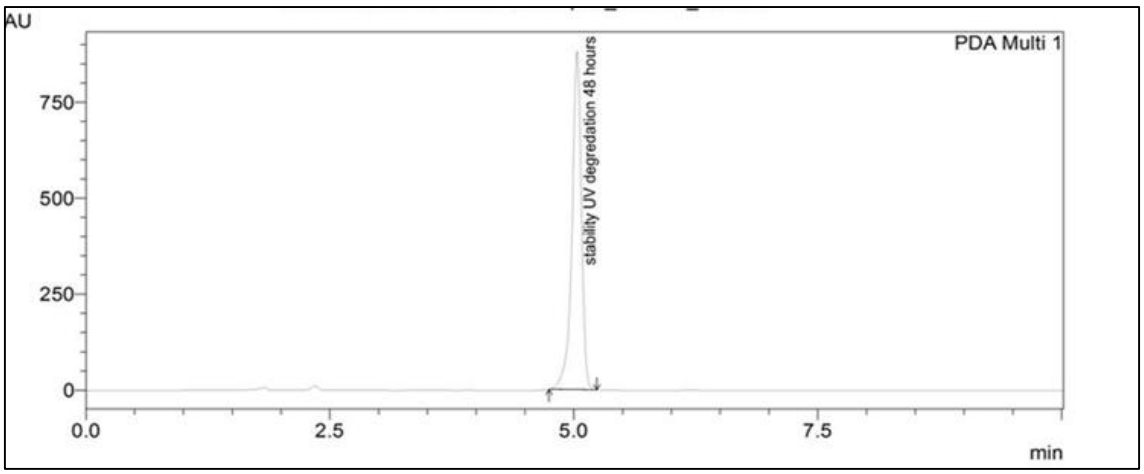

Fig. 13.The chromatogram of tolvaptan in UV degradation study after 48 hours

7. CONCLUSION

The UV-Visible spectroscopy method for quantitative estimation of bulk and tablet tolvaptan was developed and validated according to ICH guidelines. The maximum absorbance was observed at 266 nm. The wavelength 266 nm was selected for further analysis of tolvaptan.

The calibration curve was determined using drug concentrations ranging from 20-100 µgm/ml.

The RP-HPLC method for quantitative estimation of bulk and tablet tolvaptan was developed and validated according to ICH guidelines. The method was developed by selecting a detection wavelength of 266 nm. The most optimized peak was obtained with mobile phase acetonitrile: water (45:55). The optimized chromatographic conditions were a C18 column with a run time of 10 min and a retention time of 4.7 min the most resolved tolvaptan peak was obtained at a flow rate of 1 ml/min.

This developed RP-HPLC method has been validated according to ICH guidelines in terms of suitability, linearity, precision, accuracy, repeatability, and system stability studies. System suitability was achieved by injecting a standard solution containing 200 µg / ml of tolvaptan in six repetitions. For two of them, the asymmetric peak was <1.5 and the theoretical number of plates was> 2000 and the% RSD of tolvaptan was less than 2. The result indicates that the suitability parameter of the system was within the acceptable limit. All validation parameters were within acceptable limits according to ICH guidelines. Degradation studies in tolvaptan solution were conducted using a developed method. The developed RP-HPLC method has been successfully applied for the quantitative estimation of tolvaptan in the commercial tablet formulation. From the above results, it was concluded that the developed UV and RP-HPLC methods are precise and accurate and can be applied for the quantitative estimation of tolvaptan from bulk and tablet dosage forms. The method can be used for routine testing of tolvaptan by the pharmaceutical industry.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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