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Method Development and Validation for Assay of Tolvaptan IR Tablets of Multiple Strength's by Using RP-HPLC Technique

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ABSTRACT:

The developed method was a simple, efficient, economical method for the Validation of Tolvaptan and its related substances in Drug product by RP- HPLC. The separation of the standard peak from baseline achieved by using column Hypersil BDS,150×4.6mm,5um (250 x 4.6 mm, with 5µm) at an ambient temperature with a flow rate of 1.5 mL/min with isocratic elution mode. The mobile phase pumps the sample injection of 20µL at an ambient temperature. The PDA detector was set at a wavelength of 254 nm for the detection of sample for analysis for a run time of 8 min. All the parameters used in this method were validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool. Parameters are like Linearity, Specificity, Accuracy, System suitability, Robustness and Method precision.The results obtained were well within the acceptance criteria.

Key words: Tolvaptan, HPLC Method development and validation, Forced degradation and ICH guidelines

1. Introduction:

Tolvaptan is chemically known as 4-Amino-5-chloro-2,3-dihydro-N-[1-(3-methoxypropyl)-4- piperidinyl]-7-benzofurancarboxamide structurally (shown in fig 1)and its empirical formula is $C_{26}H_{25}ClN_2O_3$. It consists of Molecular weight 448.94 and present in a white to off white crystalline powder and soluble in benzyl alcohol and methanol, practically insoluble in water and melting point was approximately 224°c.Tolvaptan serves as a non-peptide antagonist targeting the vasopressin V2 receptor and is primarily prescribed for addressing euvolemic or hypervolemic hyponatremia. 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. Literature survey reveals few UV spectrophotometric, RPHPLC, LC/MSMS, UPLC and HPTLC methods for Tolvapatan estimation in bulk and pharmaceutical dosage form. So, the present proposed method developed and validated by using RP-HPLC method would serve as a simple, rapid, accurate, reliable and precised method for the determination of Tolvaptan in Bulk and pharmaceutical dosage forms.
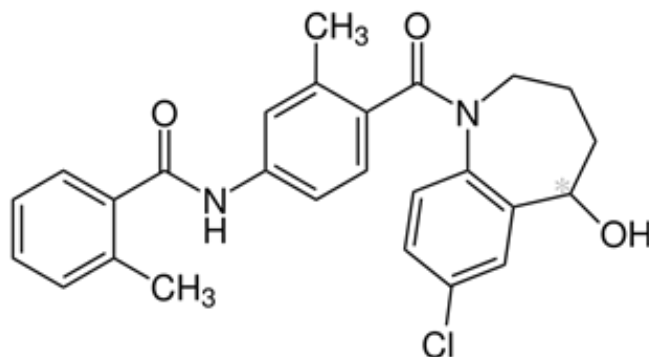

Figure.1.Structure of Tolvaptan

2.Materials and Methods:

2.1. Standard:

Tolvaptan API (Standard obtained from vendors), sample (Samples obtained from different batches of manufacturing in Neuheit pharma Ltd.)

2.2. Chemicals:

HPLC grade solvents like, Methanol, Acetonitrile are purchased from Rankem, AR grade reagents like Orthophosphoric acid, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride are purchased from Merck and Ultrapure water form Rephile.

2.3. Instruments:

Semi micro balance (Radwag, XA 82/220/2X), Pan balance (Radwag, PS 600.R2) , pH meter (Lab India, PICO+), Milli Q water system (Millipore elix – gradient),0.45 úm mdi membrane filter(Advanced micro devices), Ultra sonicator (PCI analytics),UV visible spectrophotometer(Perkin Elmer, lambda 25), HPLC (Waters Allaince separation module 2695 detector : PDA 2996/UV 2487 along with operating software Empower).

2.4.P reparation of Reagents:

2.4.1. Diluted Orthophosphoric Acid Preparation procedure:

Dissolve 5 ml of orthophosphoric acid (85%) in distilled water up to 100 ml and mix well.

2.4.2.Buffer Preparation:

Weigh 2 g of sodium dihydrogen phosphate was transferred in to 1000 mL (10 mM) of HPLC water and pH was adjusted to 3.0 with dilute phosphoric acid, then filtered through 0.45 µm nylon membrane filter and sonicated for 20 min.

2.4.3.Preparation of mobile phase:

Mix a mixture of above buffer 2000mL (50 %), Acetonitrile 1600mL (40 %) and 400mL of Methanol HPLC (10 %) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4.4.Standard Solution Preparation:

Accurately weigh and transfer 33mg of Tolvaptan Working standard into a 50mL volumetric flask add about 35mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 5ml of the above stock solution into a 100mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter

2.4.5.Sample Solution Preparation:

Weigh 5 Tolvaptan Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 33 mg of Tolvaptan into a 50mL volumetric flask. Add about 35mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 15 ml of the above stock solution into a 100ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.4.6.Preparation of placebo solution:

Weighed and transferred 234.57mg into 50ml volumetric flask.25ml of diluent was added and sonicate for 30monutes with intermediate shaking to dissolve. Made up to the volume with diluent and mixed well.Pipette 6ml of above as such sample stock solution into a 25ml volumetric flask, to this added 0.35ml of each individual impurities stock solution, Made up to the volume with diluent and mixed well. Then filtered through 0.45 Nylon membrane filter, by discarded intial 2-3ml of filtrate. Then collect the sample In HPLC vial and injected in HPLC System.

2.4.7.Optimized Chromatographic conditions:

After several trails with sodium dihydrogen phosphate buffer (optimized condition was achieved by changing pH of the buffer from pH7.8 to pH 3.0 pH was adjusted with dilute orthophosphate). The separation of the standard peak from baseline achieved by using column Hypersil BDS,150×4.6mm,5um (250 x 4.6 mm, with 5µm) at an ambient temperature with a flow rate of 1.5 mL/min with isocratic elution mode. The mobile phase pumps the sample injection of 20µL at an ambient temperature. The PDA detector was set at a wavelength of 254 nm for the detection of sample for analysis for a run time of 8 min.

3. Results and Discussion:

Method validation: Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

3.1. System Suitability:

System suitability is an integral part of chromatographic system. The calculation and comparison of verified resolution, capacity factor, tailing factor, and theoretical plate count with standard specification of system. the column was equilibrated with mobile phase for 30min with flowrate1.5mL/min and Tolvaptan standard with 400ppm concentration was injected sixes times into HPLC system after the injecting of one blank.

Table.1.System suitability Data of Tolvaptan

Parameters	Retention Time	Tailing Factor	Plate Count	Avg.Area	%RSD
Results	5.771	1.1	7311	962578	0.4

Acceptance Criteria: When calculating the peak area of Tolvaptan using six injections of the standard solution, the %RSD should not exceed 0.85. The USP tailing factor should not be greater than 2.0.Column efficiency should be more than 2000 theoretical plates are required on column.

Observation: The % RSD for the area of Tolvaptan peaks obtained from the six replicate injections of standard solution was 0.4. The USP tailing factor for Tovaptan peak was 1.1. The column efficiency of Tolvaptan peak was 7311.The retention time of tolvaptan is 5.771 mins.

Conclusion: System suitability parameters were acceptable and met the acceptance limits.

3.2. Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The determination of the excipients effect and other additives which are present in formulation can be determined by using analytical method i.e. specificity. Prepared placebo and blank are injected into HPLC system along with a standard of 400ppm concentration.
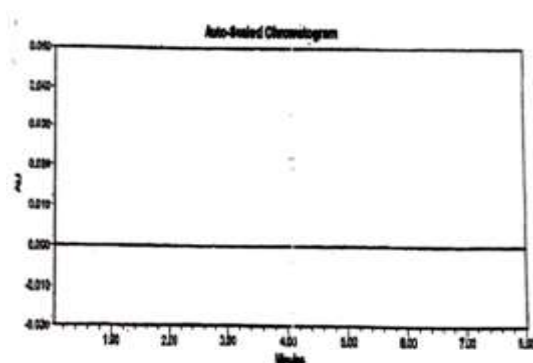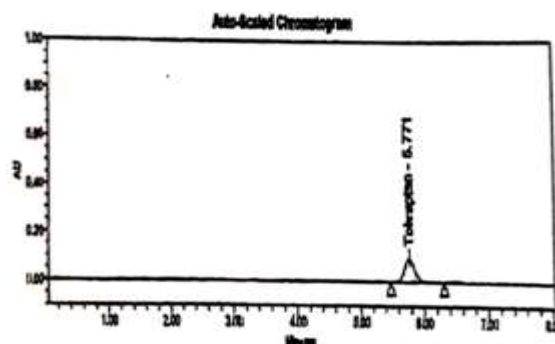

Figure.2. Chromatogram of Blank

Figure.3.Chromatogram of Tolvaptan Standard

Table.2.Specificity Data of Tolvaptan

Standard ID	Retention time(RT)	Area
Standard 1	5.794	881512
Standard 2	5.794	881737
Standard 3	5.793	882019
Standard 4	5.793	884151
Standard 5	5.793	885008
Standard 6	5.794	885647
Avg.Area	883346	
Std Dev	1812.1	
%RSD	0.2	

Table.3.Interference table for Tolvaptan

Injection ID	Name of Impurities	Retention time(t_r)	Resolution	Purity angle	Purity Threshold	Interference (Yes/No)

Blank	Tolvaptan	Not Detected	NA	NA	NA	NO
Placebo	Tolvaptan	Not Detected	NA	NA	NA	NO
Standard	Tolvaptan	5.771	NA	0.077	0.297	NA
Individual Impurities (1%)	Amino hydroxy Impurity	2.294	NA	0.561	0.635	NO
	Amino Impurity	2.930	NA	0.184	0.599	NO
	Deschloro Impurity	3.298	NA	0.854	1.123	NO
	Desmethyl 2 Impurity	4.874	NA	0.834	60.666	NO
	Desmetyl 1 Impurity	5.188	NA	0.582	1.839	NO
	Bromo Impurity	6.426	NA	0.245	0.534	NO
	4 Methyl Anologue Impurity	6.983	NA	0.181	0.782	NO
	Keto Impurity	7.935	NA	0.550	0.907	NO
Sample	Tolvaptan	5.787	NA	0.065	0.291	NO

Acceptance Limits: Each peak needs to be properly resolved. Do not interfere with the Tolvaptan peak of other peaks. NLT 0.99 is the recommended maximum purity for Tolvaptan.

Observation: There was no interference observed at the retention time of Tolvaptan due to blank and Placebo.

Conclusion: Due to no interference with retention time of Tolvaptan peak. This method is considered as specific to Tolvaptan.

3.3. Precision:

Precision of the method refers to the reproducibility of value on repeated measurements. And also define as closeness of agreement between a series of measurements obtained from multiple sampling of similar homogenous sample under the prescribed condition. Since all the test product strengths i.e., 15 mg,30 mg,45 mg.60 mg & 90 mg are dose proportional. So method precision was showed only on lower strength 15 mg and higher strengths by preparing six replicate sample preparations at 100% target level as per below preparations.

Table.4.Precision Data for Tolvaptan

S.No	Sample		Standard	
	15mg (%Assay)	90mg (%Assay)	15mg (%Assay)	90mg (%Assay)
Inj-1	99.9	100.4	100.4	99.9
Inj-2	101.7	100.9	100.9	101.7
Inj-3	98.1	100.5	100.5	98.1
Inj-4	99.0	100.5	100.5	99.0
Inj-5	98.3	101.1	101.1	98.3
Inj-6	101.8	101.7	101.7	101.8
Average	99.8	100.9	100.9	99.8
STD Dev	1.6	0.5	729.0	576.4
%RSD	1.6	0.5	0.1	0.1

Acceptance criteria: The % RSD should not be more than 2%

Observation: The above results show that % RSD for assay of Tolvaptán for six replicate sample and standard preparations of method precision was found less than 2.0%.

Conclusion: Precision data was within acceptance criteria of NLT 80% or %RSD below.

3.4. Linearity:

Linearity of an analytical method is carried out to demonstrate that concentration of an analyte is directly proportional to the peak area of analyte. Injected each level in to the chromatographic system and measured the peak area. Plotted a graph of peak area versus concentration (on x-axis concentration and on y-axis peak area) and calculated the R^2.The linearity was determined by injecting the LOQ, 50%, 80%, 100%, 120%,150% ,200% of spiked solutions.

Table.5.Linearity Data of Tolvaptan

S. No	% Level	Concentration	Peak Area
01	50	14.910	462158
02	80	23.856	742002
03	100	29.820	920694
04	120	35.784	1111348
05	180	53.676	1675645
06	200	59.640	1847638

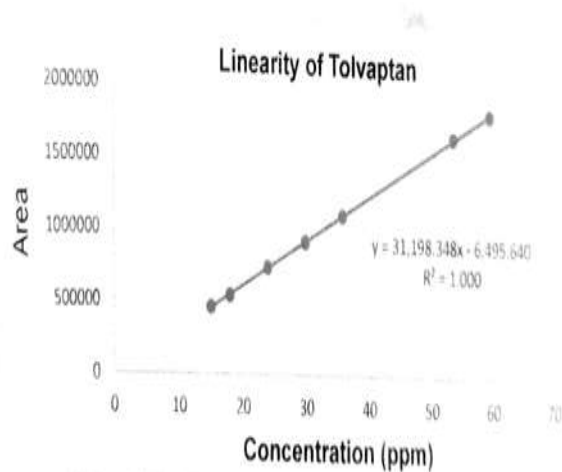| Correlation Coefficient	1.000		
Regression Coefficient	1.000		
Slope	31198.35		
Y-Intercept	-6495.64		
Bias at 100%	-0.71		
Residual sum of Squares	111207741.485		

Figure.4.Linearity Curve of **Tolvaptan**

Acceptance criteria: the correlation coefficient value should not be less than 0.999.

Observations: correlation coefficient between tolvaptan concentrations and peak areas was calculated by linear regression and was found to be 1.000 bias -0.71, slope-31198.35, residual sum of squres111207741.485.

Conclusion: correlation coefficient between tolvaptan concentration and peak area was calculated by linear regression and was found to be within the acceptance criteria.

3.5. Recovery:

Recovery of the analytical method is the capability of method to determine the correct assay value. Accuracy/ Recovery of analytical method can be determined by adding the known amount of standard drug to placebo preparation and then same shall be analysed by proposed method. The accuracy of the method was determined by calculating recovery of tolvaptan by adding additional standard in different levels as 50% to 150% which corresponds to 15 ppm to 50 ppm of target concentration of Tolvaptan API (about 30 ppm) of the target assay to placebo.

Table.6.% Recovery Data of Tolvaptan

S.No	Recovery level	Sample	Added "ppm"	Found "ppm"	% Recovery	Average % recovery	%RSD
1	50%	Sample-1	14.98	15.11	100.8	100.6	0.2
		Sample-2	15.00	15.05	100.4		
		Sample-3	14.99	15.06	100.5		
2	100%	Sample-1	29.94	29.74	99.3	99.5	0.2
		Sample-2	29.94	29.81	99.6		
		Sample-3	29.95	29.86	99.7		
3	150%	Sample-1	55.90	54.00	100.2	100.2	0.3
		Sample-2	53.90	53.95	100.1		
		Sample-3	53.89	53.98	100.2		

Acceptance criteria: The mean % recovery of the standard and sample at each level should be not less than 98% and not more than 102%.

Observation: The % Recovery of Tolvaptan was found to be 100.1%

Conclusion: The recovery results indicate that the test method has an acceptable level of accuracy. The results were found to be within the limits.

3.6. Robustness:

The robustness of analytical method is a measurement of its capacity to remain unaffected by small changes but deliberate changes in procedure parameters and provides an indication of its reliability during normal usages. Such as Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Influence on variation of flow rate ($\pm$0.2 mL/min), column temperature ($\pm$5°C), Organic ratio ($\pm$5%)and pH ($\pm$0.2) on developed method.

Table.7.Robustness results for Tolvaptan

Name of the Parameter	RT	Avg Area	%Assay	%RSD	Theoretical Plates	Tailing factor
low flow rate (1.3 mL/min)	5.927	1076119	100.8	0.2	5007	1.2
High flow rate (1.7 mL/min)	4.551	826514	101.7	0.2	4504	1.2
Low Column Temp(23ºc)	5.166	921747	101.6	0.2	4807	1.2
High Column Temp(30ºc)	4.814	928169	101.7	0.2	5128	1.2
	NA	NA	NMT 2.0	NMT 0.85	NLT 2000	NMT 2.0

Acceptance Criteria: The % recovered amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%

Observation: System suitability met the acceptance criteria in Robustness parameters hence method is Robust.

Conclusion: The test method was found to be robust against changes in flow rate, buffer pH, column temperature, and organic ratio.

3.7. Filter Compatibility:

Filter validation is the process of confirming that the filter used to sterilize a pharmaceutical product does so adequately, by efficiently removing microorganisms. Filter compatibility was performed by preparing standard and sample solutions and comparing the results for unfiltered and filtered solutions. Unfiltered samples are obtained by centrifuging samples at 5000 rpm for 5 minutes and collecting clear supernatant .Compatibility of 0.45µm nylon filter and PVDF filter was studied. Standard and dissolution samples were filtered and analyzed along with unfiltered solutions. The % assay and difference were calculated.

Table.8.Filteration Data for Tolvaptan

Filters	Unfiltered	0.45µ nylon	0.45µ PVDF	0.45µm PTFE	Acceptance limits
Recovery	101.5	100.4	101.7	101.4	The Difference in % Assay of unfiltered and Filtered samples should not be more than ± 2.0.
Difference		1.1	0.2	0.1	

Conclusion: After the analysis it was found that nylon filter was suitable for filtration as the filter interference observed is below ± 2%. Precision data at Q point time was within acceptance criteria of NLT 80% and RSD below 5%

3. 8. Stability solution:

To obtain accurate and reliable results, it is important to keep the test solutions, indicators and reagents used in HPLC methods for a specific period of time, such as a day, a week or a month, according to special requirements. These methods aim to ensure the proper maintenance of the workload. Stability of solution was determined by injecting prepared standard and sample solutions into an HPLC system at initial stage, with freshly prepared standard after a period of time. Tolvaptan standard was prepared and injected into the HPLC system at initial stage, day-1, day-2 and day-5 (with freshly prepared standard). Similarity factor for initial standard, day 1, day-2 and day-5 standard area response of Tolvaptan peak from the standard preparation.

Table.9. Solution Stability data of Dexemedetomidine

Day	Conditions	Standard			Samples	
		Weigh	Area	Similarity Factor	% Assay	Similarity Factor
1	Fresh	29.82	920131	----	100.4	----
	Room temperature (25+3°C)	29.91	926088	1.00	99.1	1.1
	Refrigerator (2-8°C)	29.91	925102	1.00	99.5	0.9
2	Fresh	29.82	922751	----	----	----
	Room temperature (25+3°C)	29.91	833542	1.01	99.1	1.3
	Refrigerator (2-8°C)	29.91	933478	1.01	99.1	1.3
5	Fresh	29.65	899906	----	----	----
	Room temperature (25+3°C)	29.91	930148	1.01	100.1	0.3
	Refrigerator (2-8°C)	29.91	919693	1.02	99.9	0.5

Acceptance Criteria: The % standard deviation from the peak area is limited to a maximum of 2.0.

Conclusion: From the observations, it was concluded that this pattern is stable for 48 hours at room temperature (25 ± 3ºC) and refrigerated (2ºC to 8ºC).

4. Forced degradation Studies:

The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, The ICH definition of stress testing for the drug product is "studies undertaken to assess the effect to severe conditions on the drug product. Such studies include photo stability testing and specific testing on certain products like metered dose inhalers, creams, emulsions etc. As per FDA guideline "Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods".

4.1. Thermal Degradation:

Stress testing is likely to be carried out on single batch of the drug substance (API). Thermolytic degradation may lead to hydrolysis / dehydration / isomerization / epimerization / decarboxylation / rearrangements and some kinds of polymerization reactions. ICH guidelines suggest that thermolytic degradation study should be carried out at temperatures (in 10 increments e.g. 50ºC, 60ºC, etc.) above that for accelerated testing and withdraw the sample at different time intervals during reaction condition. If reasonable degradation (i.e. 5-20%) has seen, testing can be stopped at this point.

4.2. Photolytic Degradation:

The photochemical stability of the drug was studied by exposing the 100µg/ml solution to UV light by keeping the beaker in UV chamber for 24hours. For HPLC study, the resultant solution was injected into the system and the chromatogram were recorded to assess the stability of sample.

4.3. Acidic Degradation:

Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N HCl then kept in oven at 60^0c for 1 hour then cool and add 1 ml of 0.1N NaOH it then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

4.4. Base Degradation:

Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N NaOH then kept in oven at 60°C for 1 hour then cool it and add 1 ml of 0.1N HCl then make up the volume up to 50ml with mobile. Phase, then place the sample in the vial and measure the chromatogram

4.5. Peroxide Degradation:

Sample solution (100µg/ml) and 1 ml of 20% hydrogen peroxide (H_2O_2) was mixed. For HPLC study, 100µg/ml was injected into the system and the chromatogram was recorded to assess the stability of sample.

4.6. Control Degradation:

Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then kept in oven at 60^0c for 4 hour then cool and then place the sample in the vial and measure the chromatogram.

Table.10.Forced degradation Data of Tolvapatan

S.No	Sample Name	Degradation Conditions	Purity Angle	Purity Threshold	% Assay	% Degradation	Purity (Pass/Fail)
1	Control sample	Initial	0.067	0.266	102.1	--	Pass
2	Acid Degradation	5N HCl-5 mL at 80°C- 4 hours sample	0.194	0.398	86.3	15.8	Pass
3	Base Degradation	5N KOH-5 mL, at 80°C- 5 minutes sample	0.079	0.291	98.0	4.1	Pass
4	Peroxide Degradation	30 % H202-5 mL at 80ºC-4 hours sample	0.059	0.277	98.1	4.0	Pass
5	Photolytic Degradation	1.2mill/LUX Hours	0.052	0.250	101.3	0.8	Pass
6	Thermal Degradation	At 105°C- 7 days	0.064	0.265	100.4	1.7	Pass
7	Humidity Degradation	90% RH -7 days	0.051	0.249	102.6	0.5	Pass

Acceptance Criteria: Peak purity should be Pass and Value should be in positive

Observation: Peak purity was obtained Pass, Purity value obtained in Positive, %degradation 5.8% obtained in Acid degradation, No interference was observed with treated blank

Conclusion: The proposed method gave good resolution of Tolvaptan and its degradants. The method is confirmed to be stable indicating capability of distinguishing the active pharmaceutical ingredient (API) from any degradation (decomposition products) formed during the defined storage conditions during the stability testing period.

5. Conclusion:

The RP-HPLC method for quantitative estimation of bulk and tablet tolvaptan was developed and validated according to ICH guidelines. The separation of the standard peak from baseline achieved by using column Hypersil BDS,150×4.6mm,5um (250 x 4.6 mm, with 5µm) at an ambient temperature with a flow rate of 1.5 mL/min with isocratic elution mode. The mobile phase pumps the sample injection of 20µL at an ambient temperature. The PDA detector was set at a wavelength of 254 nm for the detection of sample for analysis for a run time of 8 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 400 µg / ml of tolvaptan in six repetitions. For two of them, the asymmetric peak 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 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.

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