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Methodological Advancements in Tolvaptan Analysis: HPLC-Based Stability Indication with LC-MS Validation

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Abstract

A stability-indicating RP-HPLC method was developed and validated for the estimation of Tolvaptan in bulk and pharmaceutical dosage forms followed by identifying the degradants obtained in stability studies by LC-MS. Grace HPLC C18 (4.6X100mm, 2.7micron) column was used with mobile phase consisting of 0.1% formic acid and methanol in the ratio of 20:80 v/v. The flow rate was maintained at 1ml/min, at 253 nm. The retention time was 4.814 minutes. The stress studies were performed as per ICH guidelines under acidic, alkali, oxidative, thermal, photostability and neutral conditions. The drug peak was well separated from the peaks of degraded products. From the degradation studies it is evident that the drug showed instability under acidic, alkali, oxidative and neutral conditions. But drug is stable in thermal and photo studies. The linearity of the method was observed in the concentration range of 5-30 µg/mL with the number of theoretical plates & tailing factor being 3486 & 1.13 respectively with a correlation coefficient of 0.999. The percentage assay of Tolvaptan was found to be 100.098%. The method was validated for its accuracy, precision and system suitability. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the estimation of Tolvaptan in pharmaceutical dosage forms.

Keywords: Tolvaptan, RP-HPLC, LC-MS, ICH Guidelines

Full-length article **Corresponding Author*, e-mail*:* kumudiniphd30@gmail.com

1. Introduction

Chemically Tolvaptan is N-[4-[(5R)-7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1-benzazepine-1-carbonyl]-3-methylphenyl]-2-methylbenzamide. Tolvaptan is a white to off white crystalline powder with a molecular weight 448.94g/mol. Tolvaptan is soluble in benzyl alcohol and methanol, practically insoluble in water and hexane. Tolvaptan melting point was approximately 2240C. Its empirical formula is C26H25ClN2O3. Chemical structure of Tolvaptan is shown in Fig.1. It is not official in any pharmacopoeia. Tolvaptan is a selective competitive vasopressin receptor-2 antagonist, the first and only oral drug in its class. It is a diuretic agent. It is used to treat Hyponatremia (low blood sodium levels) associated with congestive heart failure (CHF), cirrhosis, and the syndrome of inappropriate anti-diuretic hormone (SIADH). High levels of vasopressin can cause an imbalance that results in low sodium levels and fluid retention. Tolvaptan reduces the level of a vasopressin, and prevents vasopressin-induced re-absorption of water, by competitively blocking vasopressin binding at V2 receptors of distal portions of nephron, promoting aquaresis or electrolyte-free removal of water leading to an increase in urine volume with minimal change in the concentration of electrolytes [1,2,3,4]. According to a review of the literature, only a few numbers of analytical techniques, including UV, HPLC, UPLC, and LC-MS methods, have been documented for the quantification of Tolvaptan in biological samples, bulk, and pharmaceutical dosage forms. A quick and accurate method for determining the presence of Tolvaptan in pharmaceutical dosage forms and bulk has been developed by the author utilizing liquid chromatography. This method also entails studying and identifying degradation pathways for degradants using LC-MS. The newly developed method has been shown to be exact, sensitive, and accurate. Limits of detection, limits of quantitation, linearity, accuracy, precision, specificity, and solution stability were all assessed as validation parameters [5,6,7].

2. Materials and Methods

2.1 Instrumentation

2.1.1. HPLC-MS conditions

These analyses were performed on an Agilent 1260 Infinity II with Agilent 6540 UHD Accurate-Mass Q-TOF

LCMS. Chromatographic separation was achieved using Agilent HPH-C18, (4.6X100 mm, 2.7 micron) column maintained at room temperature. The gradient mobile phase consisted of a mixture of methanol/formic acid (80:20 v/v). The flow rate was 0.5 mL/min and the injection volume was 20 µL. Dual AJS ESI source operated in positive ion mode and analyte quantification was achieved. The ion source parameters were: nebulizer gas 35 psig; sheath gas temperature was 350°C, sheath gas flow was 11 L/min, capillary voltage 3500v, nozzle voltage 1000v, gas flow is 8 L/min and temperature 300°C. Mass range was taken from m/z 50 to 1700.

2.1.2. Chemicals and solvents

The working standard of Tolvaptan was provided as gift sample from Lupin Pharmaceuticals, Pune, India. The market formulation TOLVAT 15 from MSN Laboratories (Tolvaptan 15 mg) was procured from local market. HPLC grade water, methanol and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai, India. Formic acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai, India.

2.1.3. Preparation of standard stock solution

Tolvaptan standard stock solution was made by combining 10 mg of the drug with 10 ml of methanol to achieve a concentration of 1000 µg/mL. Additional dilutions in methanol were produced from this solution [9,10].

2.1.4. Selection of mobile phase

The standard solution of Tolvaptan (10µg/mL) was injected into the HPLC system and run-in different solvent systems. Different mobile phases like methanol and distilled water, ACN and distilled water in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Tolvaptan.

After few trials, Methanol: 0.1 % Formic Acid (80:20 v/v) was chosen as the mobile phase, which gave acceptable peak parameters [14,15].

2.1.5. Preparation of mobile phase

Mobile phase was prepared by mixing Methanol and 0.1 % Formic Acid in the ratio of 80:20 v/v. It was then filtered and sonicated for 10 min [16].

2.1.6. Preparation of sample solution

Twenty tablets with label claim 15 mg of Tolvaptan (Tolvat 15 Tablets, MSN Labortories) were measured and ground. A 10 ml volumetric flask with 5 ml of methanol and an amount of powder equal to 10 mg of tolvaptan was used. After being ultrasonically processed for 10 minutes, the mixture was filtered using Whatman filter paper 45 and the volume was made up with methanol to achieve a concentration of 1000 µg/mL. The concentration was further diluted to 10µg/ml before being injected into the system.

2.1.7. Chromatogram and system suitability parameter of drug

The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of Tolvaptan (10µg/mL) was injected on system. The retention time for the drug was found be 4.813 min. at the wavelength of 253 nm. Chromatogram of Tolvaptan is shown in Fig. 2

3. Validation of analytical method

3.1. Linearity

A solution containing 100µg/mL of methanol was made from the normal stock solution (1000µg/mL) of Tolvaptan. Additionally, a range of solutions with six different concentrations were made using this solution. By examining six solutions with concentrations ranging from 5 to 30µg/mL, the linearity (connection between peak area and concentration) was ascertained. The calibration curve's equation was discovered to be y = 135327x + 132751. The calibration curve, which is seen in Fig. 3, was created by plotting the drug's peak area against the matching concentrations [14].

3.2. Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations in linear range were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated [10,17].

3.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method [15,16].

3.4. Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 998, indicating the no interference of any other peak of degradation product, impurity or matrix [15,16].

3.5. Assay

Tolvaptan 15 tablets formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was injected and area was recorded. Concentration and % recovery was determined from linear equation [15].

3.6. Accuracy

Recovery experiments were conducted by adding three different concentrations of the reference standard to the tablet sample solution 50, 100, and 150% in order to test the method's accuracy. The sample solution's basic concentration was 10µg/ml. The linearity equation was used to calculate the recovery rate [16].

3.7. Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types.

Table 1. Summary of Validation Parameters by HPLC

Sr. No.	Validation parameters	Tolvaptan Results
1.	Linearity equation R^2 Range	y = 135327 x + 132751 R^2= 0.990 5-30 µg/mL
2.	Precision	(%RSD)
	Intraday	0.314 -0.512
	Interday	0.305 – 1.130
3.	Assay	100.098 ± 0.615
4.	Accuracy	Mean ± %RSD
	50	99.825 ± 0.545
	100	99.175 ± 0.639
	150	99.918 ± 0.915
5.	Limit of detection	0.492 µg/mL
6.	Limit of quantitation	1.492 µg/mL
7.	Specificity	Specific
8.	Robustness	Robust

Table 2. Summary of Degradation Conditions

SN	Parameter	Stress Condition	% Drug Content	% Degradation
1	Acid Degradation	2 N HCl for 2 Hr Refluxed at 60^0c	89.688	10.312
2	Alkaline Degradation	2 N NaOH for 2 Hr Refluxed at 60^0c	92.251	7.749
3	Oxidative Degradation	30% H_2O_2 for 2 hr Refluxed at 60^0c	95.046	4.954
4	Thermal Degradation	105^0C for 4 hr	99.618	0.382
5	UV Degradation	200 watthrs/sq.m	99.174	0.826
6	Fluorescence Degradation	1.2 millon lux hrs	99.451	0.543

Table 3. Summary of Degradants by acid, alkali, and Oxidation Treatment

Degradation Method	m/z ratio	Degradants	RT in Min.
Standard Tolvaptan	449	-	11.349
Acid treated Tolvatan	318	D1	7.553
	252	D2	8.601
	119	D3	9.599
Alkali treated Tolvaptan	-	D1	Absent
	252	D2	8.163
	119	D3	9.591
Oxidation treated Tolvatan	318	D1	7.566
	252	D2	8.614
	-	D3	Absent

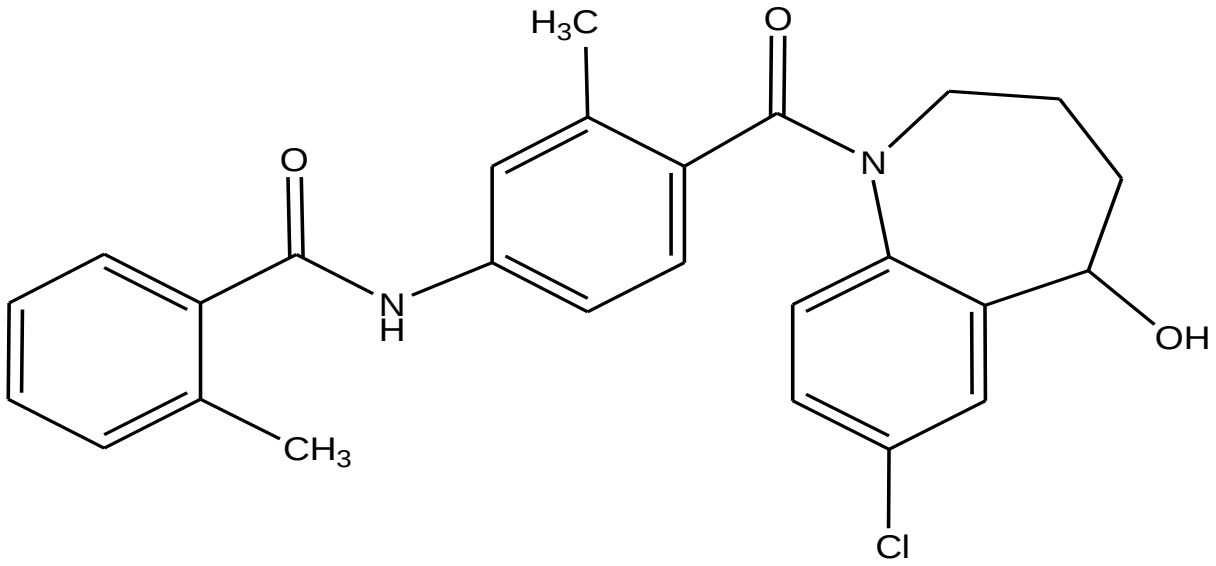

Figure 1: Structure of Tolvaptan

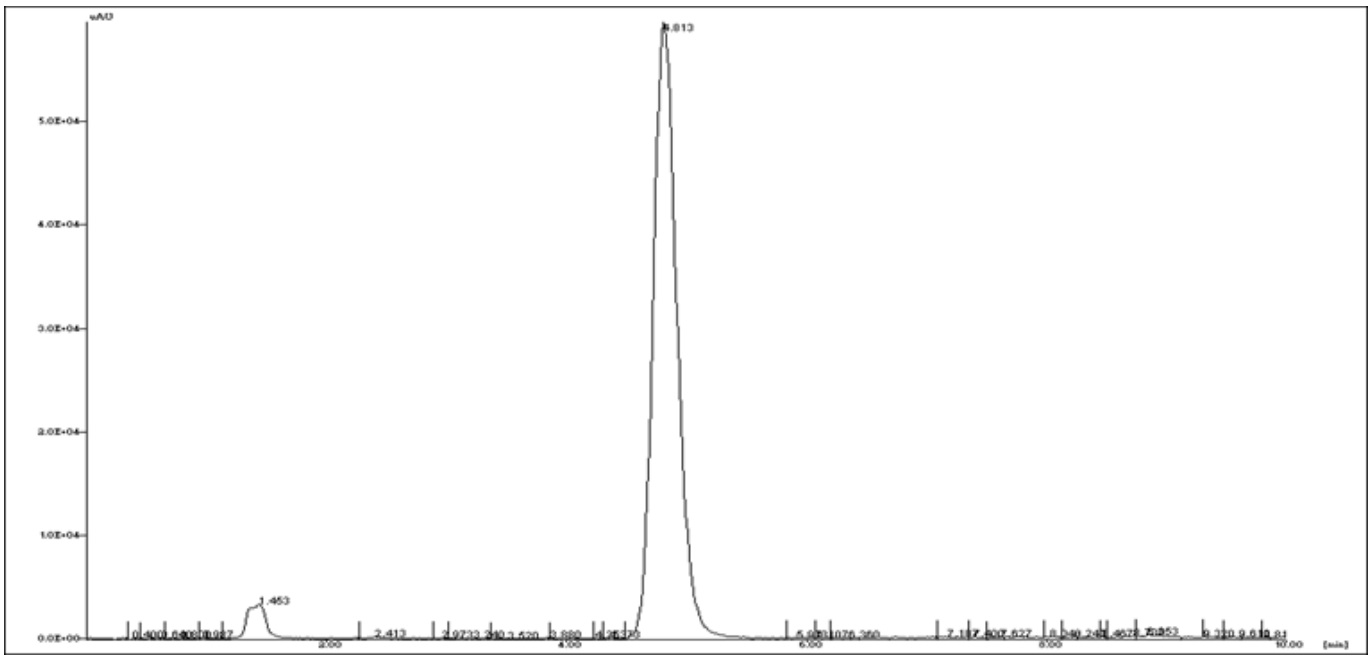

Figure 2: Chromatogram of Tolvaptan (10 µg/ml)

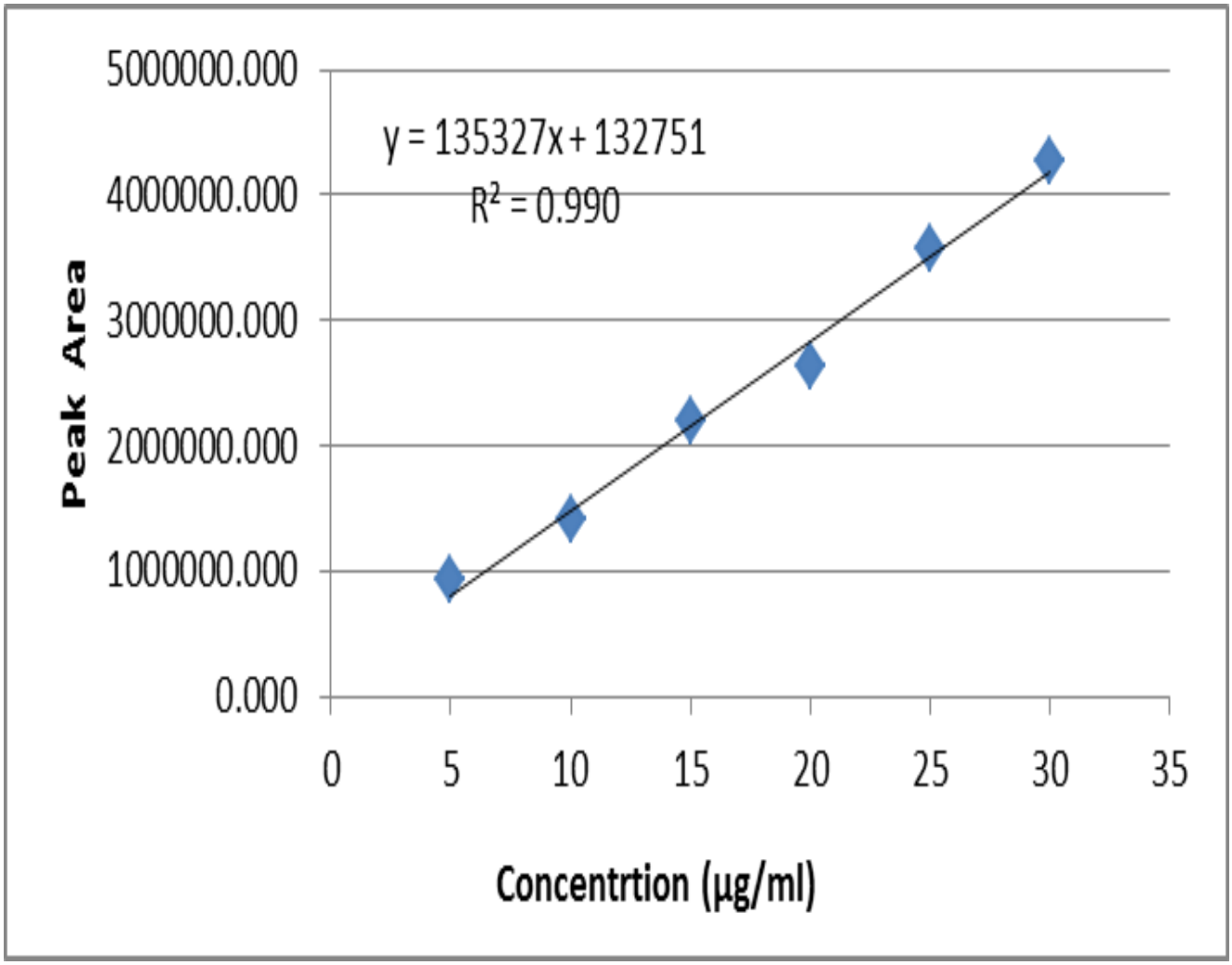

Figure 3: Linearity curve of Tolvaptan (5-30 µg/ml)

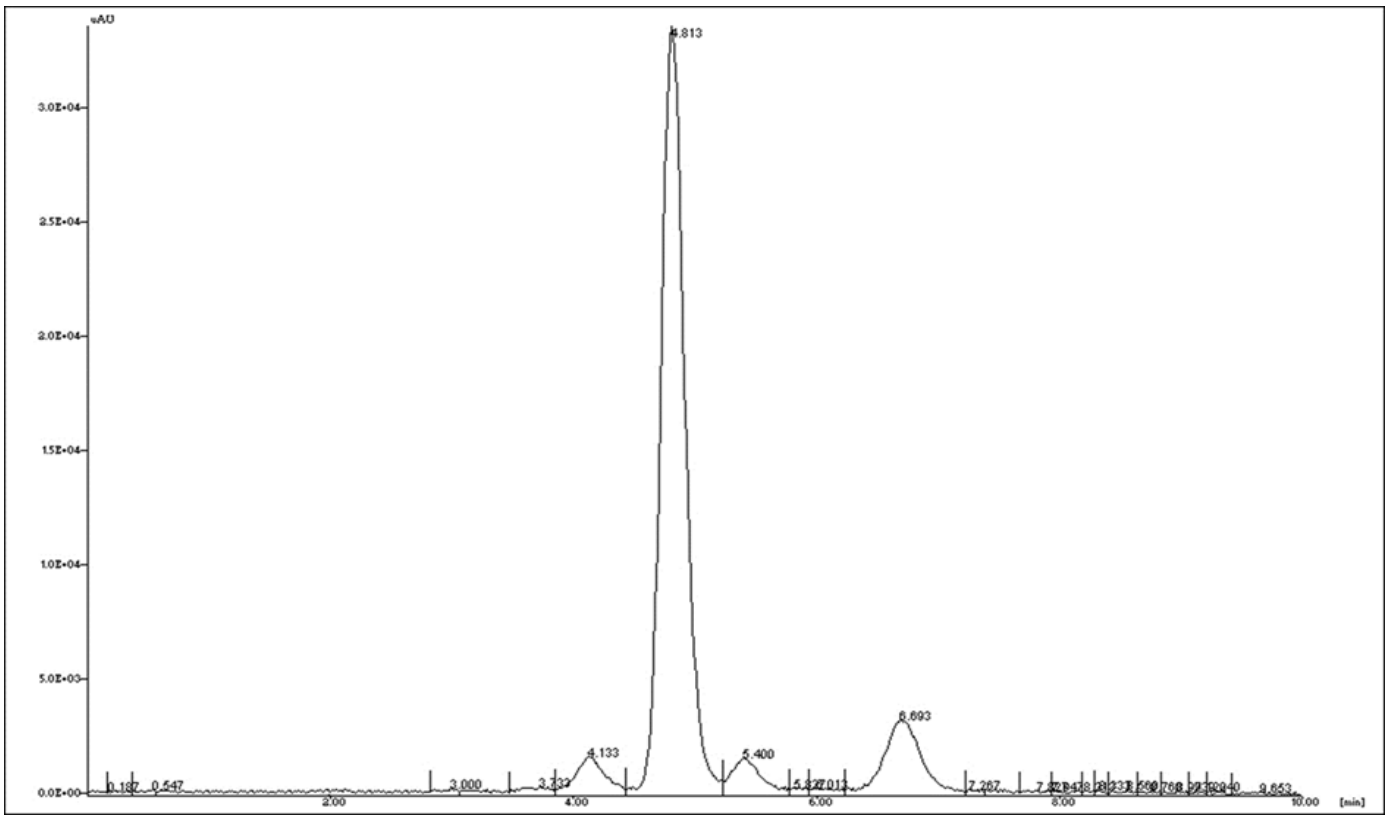

Figure 4: Chromatogram of Tolvaptan after acid degradation

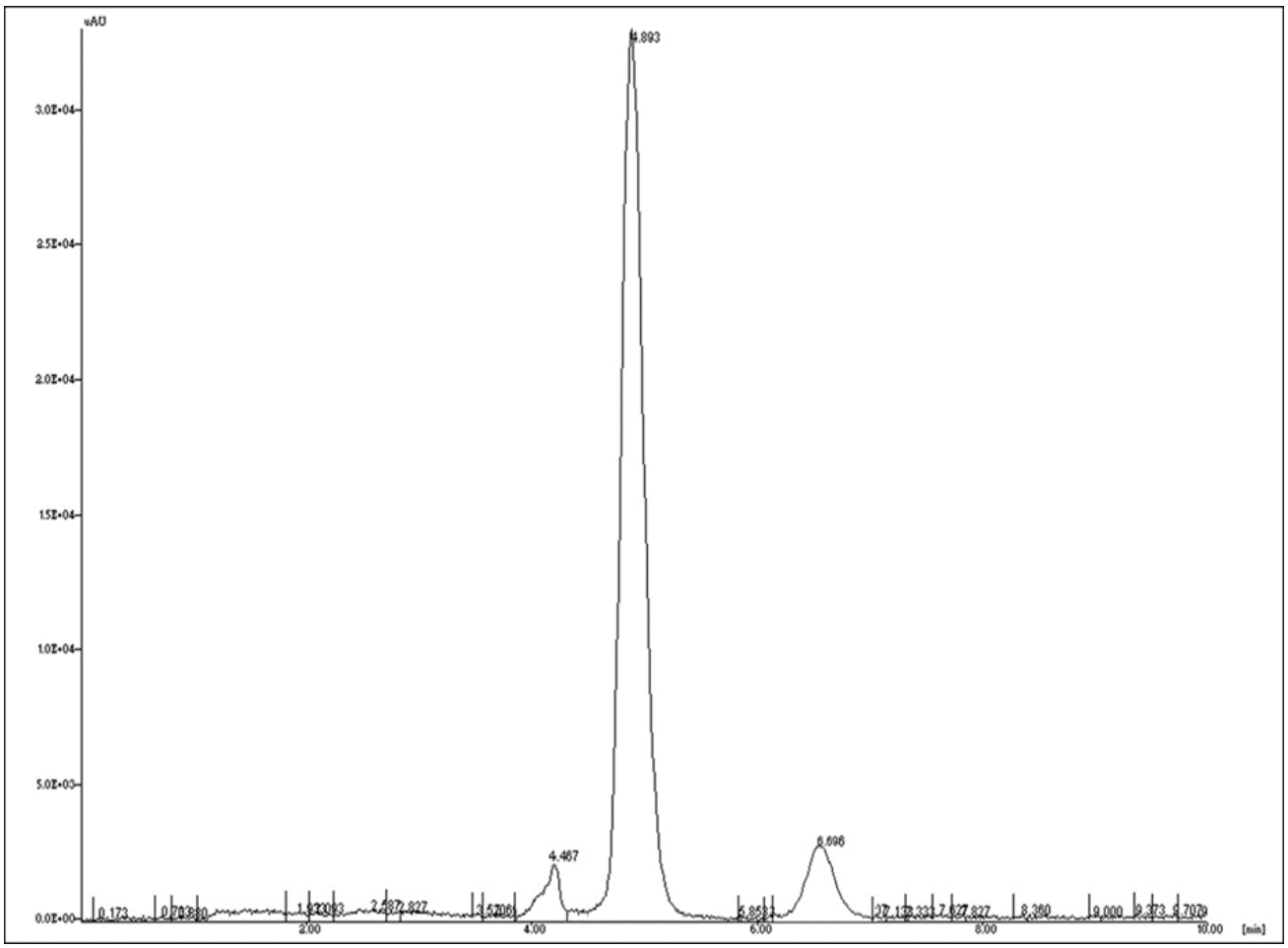

Figure 5: Chromatogram of Tolvaptan after alkaline degradation

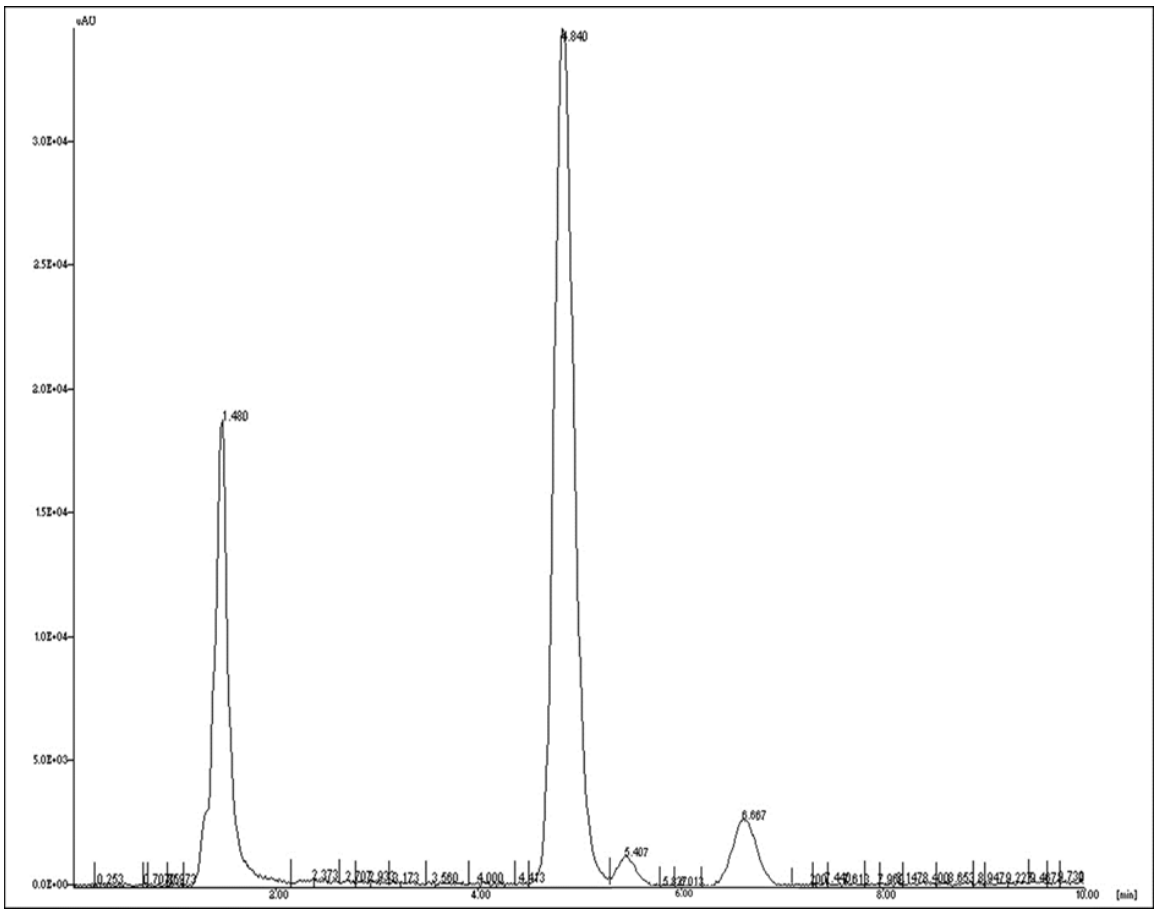

Figure 6: Chromatogram of Tolvaptan after oxidation with 30% v/v H_2O_2

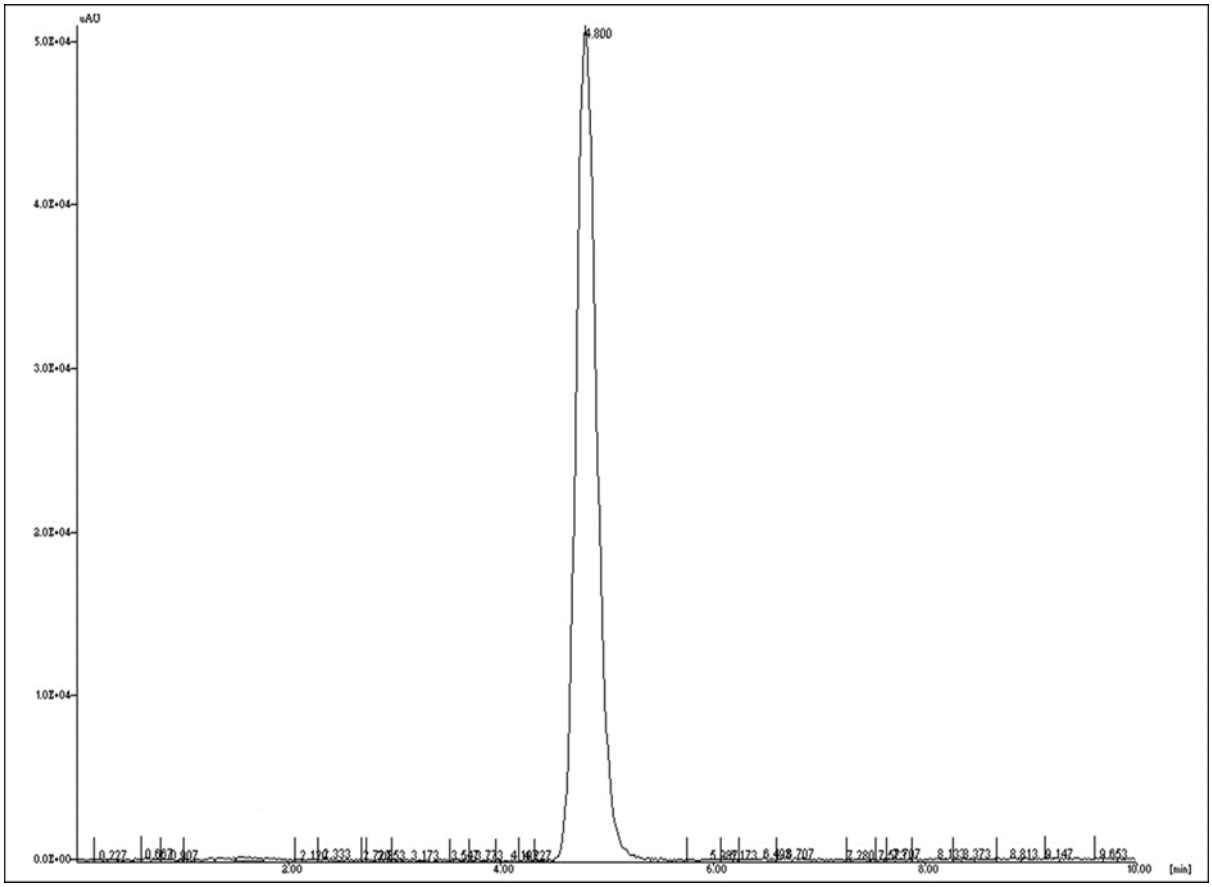

Figure 7: Chromatogram of Tolvaptan after dry heat degradation

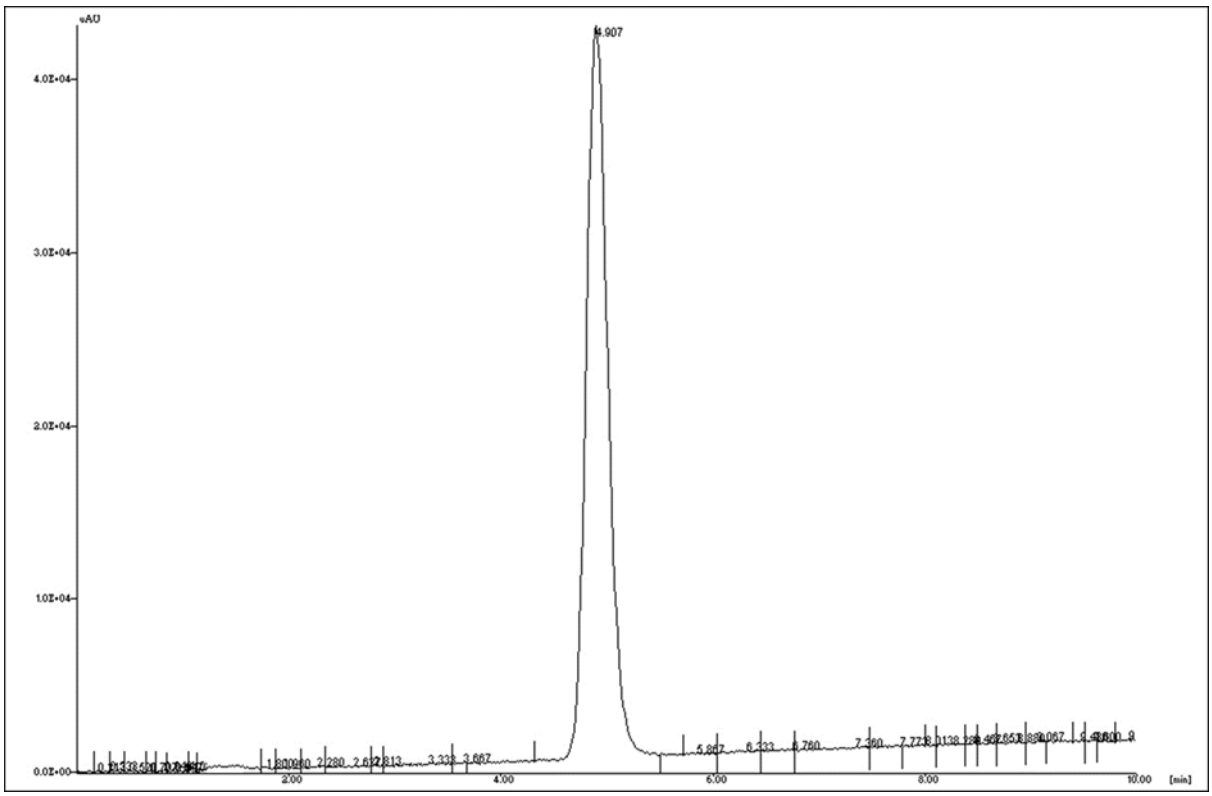

Figure 8: Chromatogram of Tolvaptan after UV illumination exposure

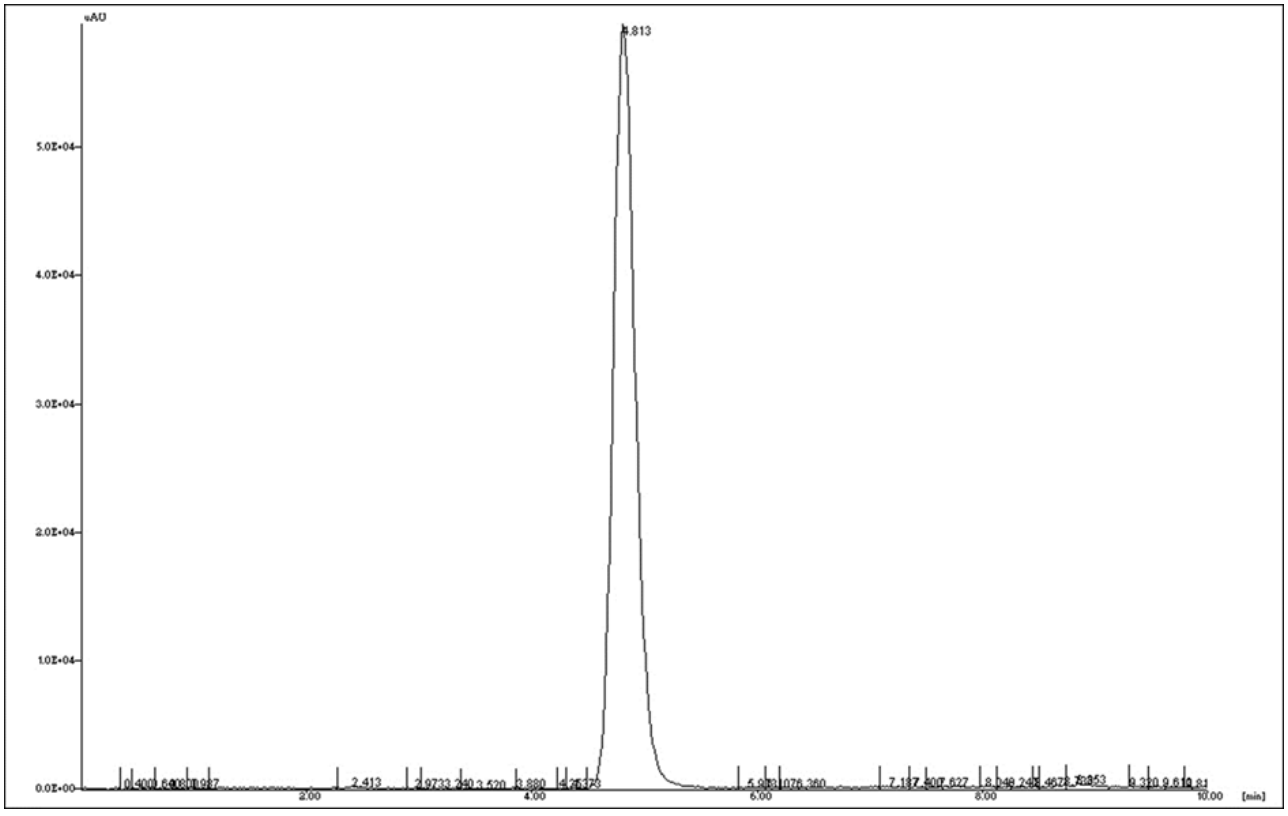

Figure 9: Chromatogram of Tolvaptan after florescent light exposure

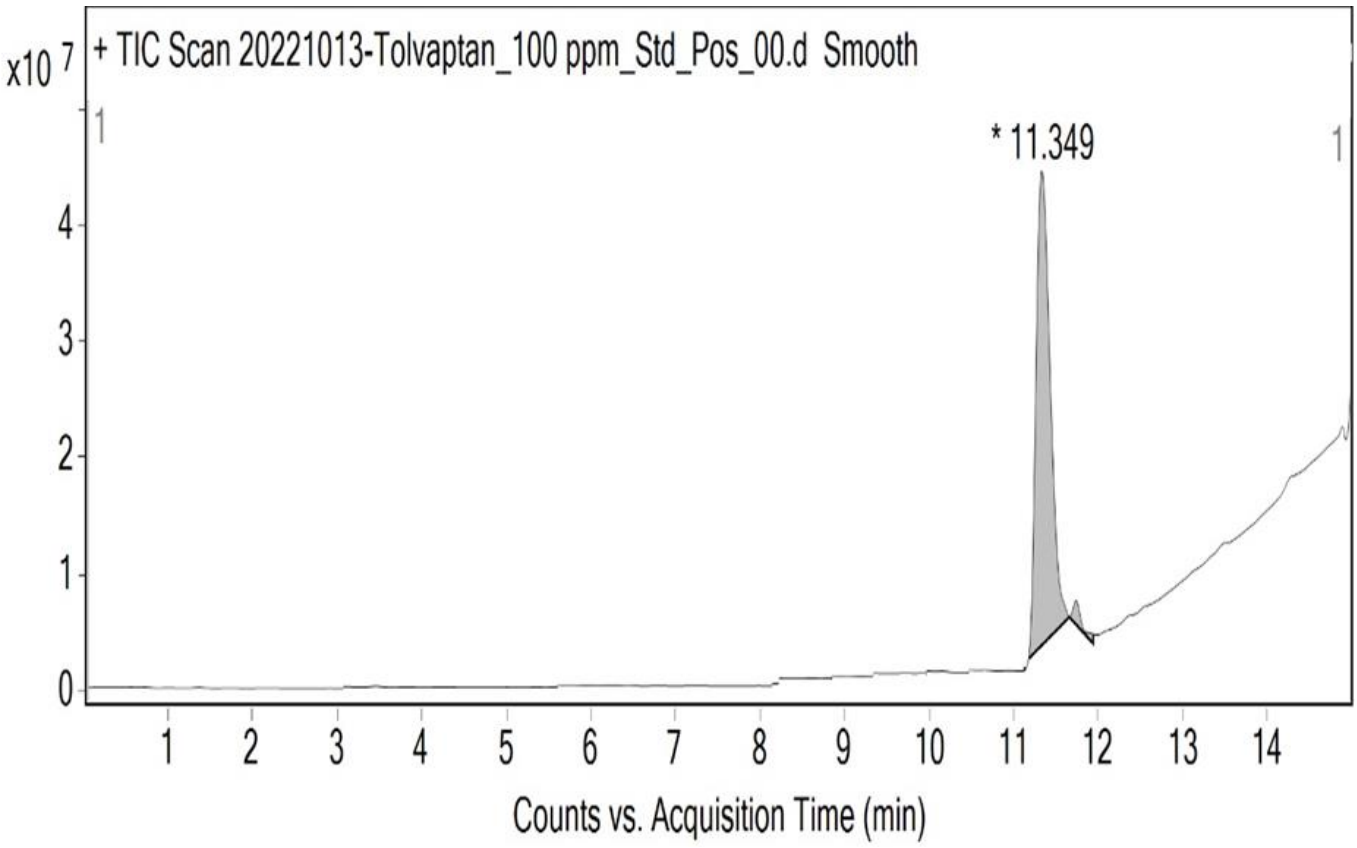

Figure 10: LC-MS Chromatograph of std. Tolvaptan

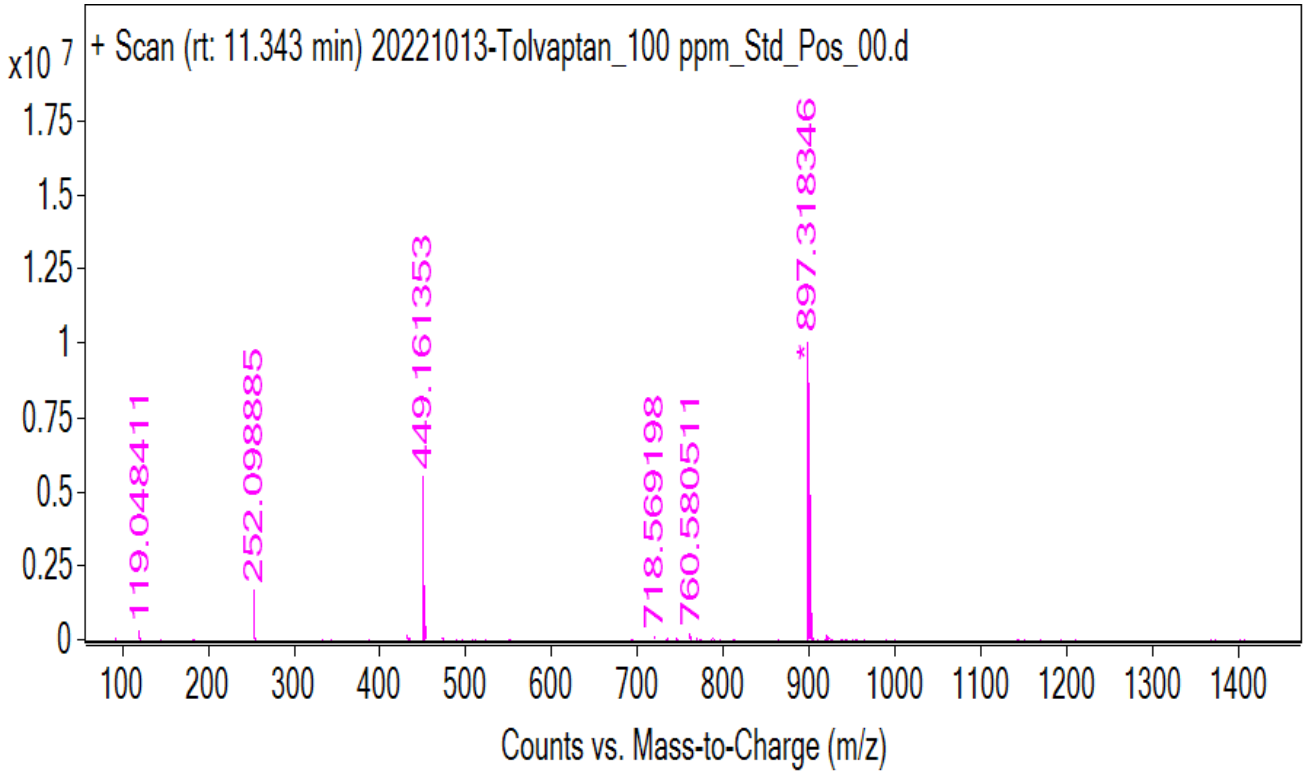

Figure 11: LC-MS mass spectrum of std. Tolvaptan (RT – 10.921 min)

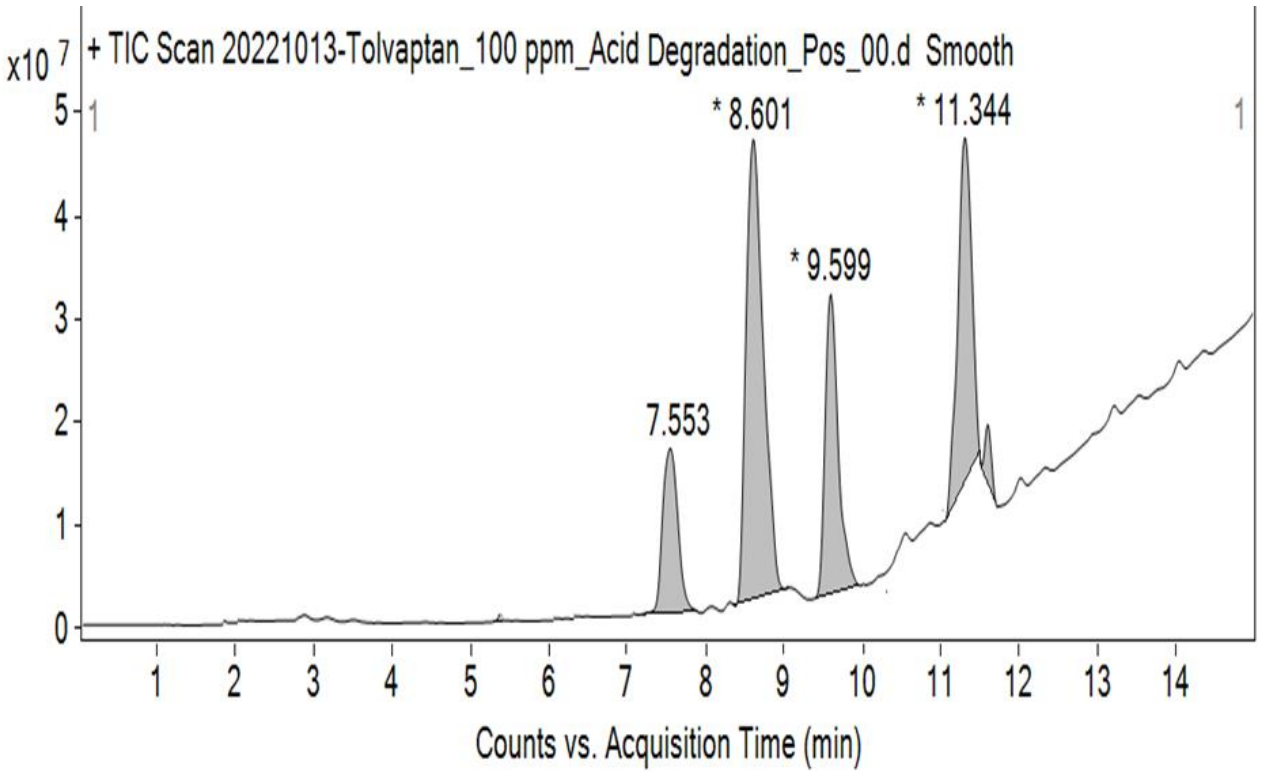

Figure 12: LC-MS Chromatograph of degradants of Acid treated Tolvaptan

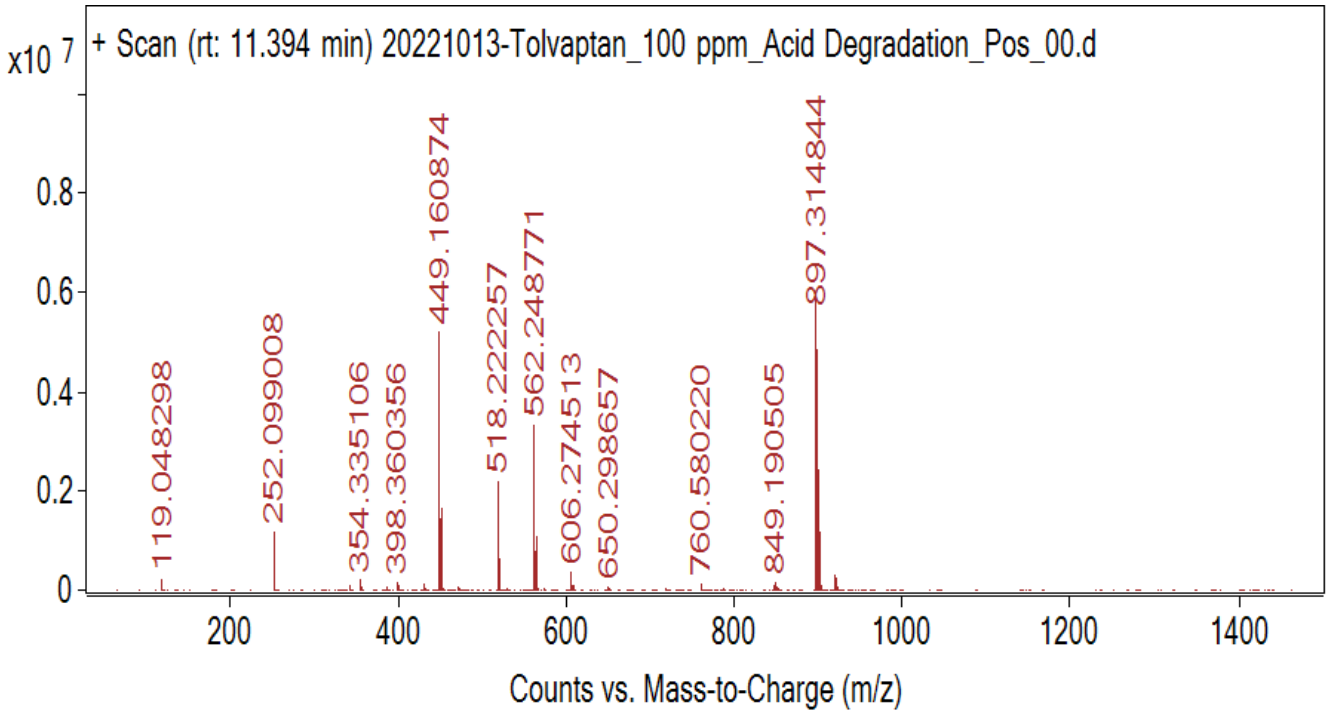

Figure 13: LC-MS mass spectrum of degradants of Acid treated Tolvaptan

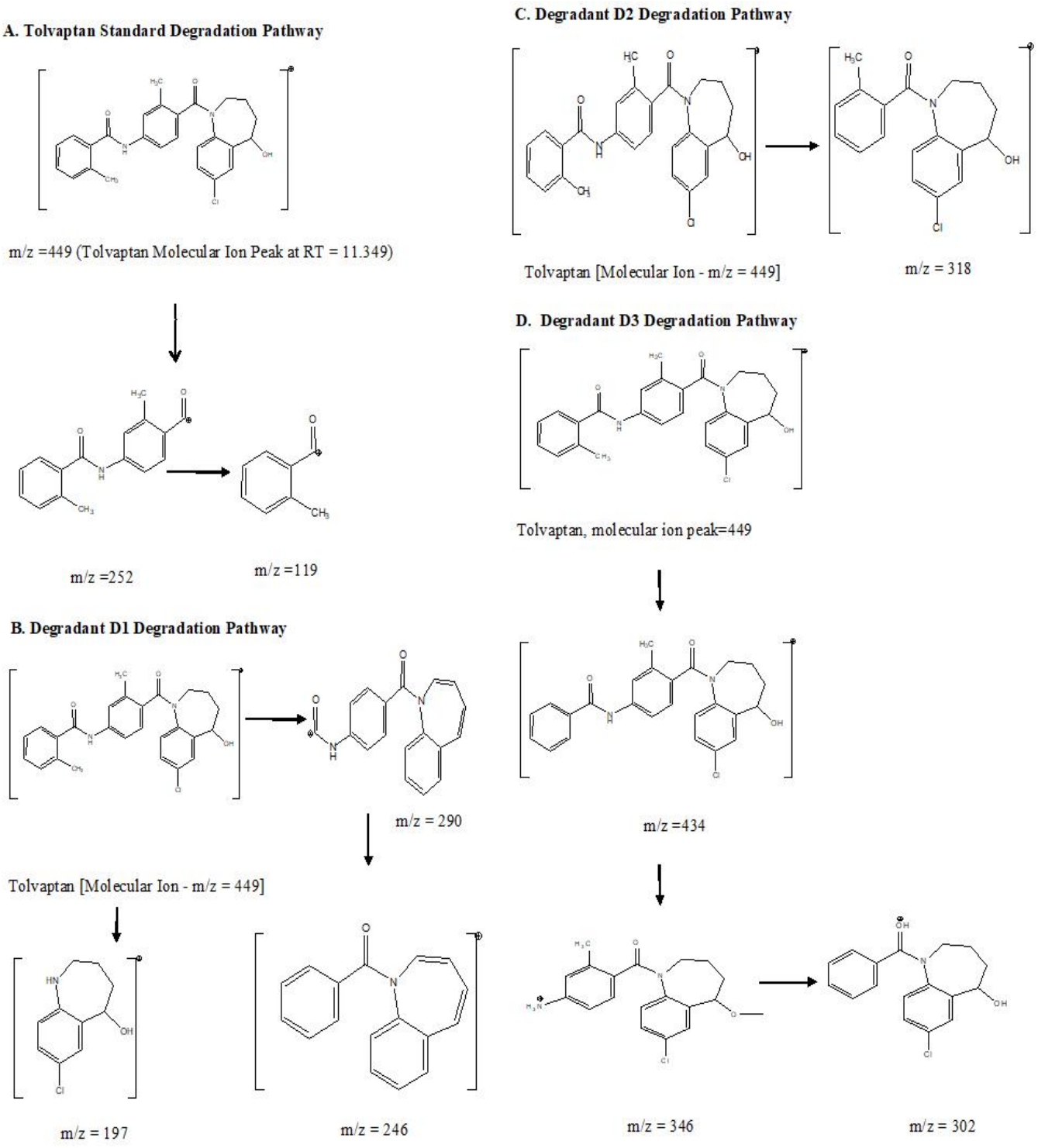

Figure 14: Proposed Degradation pathways for standard Tolvaptan as well as degradants D1, D2 and D3

The robustness of the method was determined by making slight changes in the chromatographic conditions such as flow rate and percent of composition of the mobile phase on the quantification of the drug substance and selectivity was studied [10, 16].

4. Stress degradation studies of bulk drug

In order to demonstrate how the quality of a drug changes under the effect of various environmental factors like hydrolysis, oxidation, temperature, etc., stability studies were conducted. In the solid state, photolytic deterioration and dry heat were used.

4.1. Acid hydrolysis

1 ml of 2N HCl was added to 1 mL of a drug standard stock solution (1000µg/mL), and the volume was increased to 10 mL with methanol to create a 100mcg/ml solution. The solution was refluxed for 2 hours at 600°C, neutralized with 2N NaOH, and 1 mL of the mixture was diluted to 10 mL with methanol to get 10 µg/mL of the mixture. The preparation of the acid degradation blank without analyte is same. With three peaks of degradant, the percent recovery for Tolvaptan during acid hydrolysis was 89.688%. Fig. 4 displays the typical chromatogram [16].

4.2. Alkaline hydrolysis

To 1 ml standard stock solution of drug (1000 µg/mL), 1 mL of 2N NaOH was added and volume made to 10 mL with methanol to get 100 µg/mL solution. The solution was refluxed for 2 hr at 60^0C, neutralized with 2N HCl and 1 mL diluted to 10 mL with methanol to get 10 µg/mL solution. Alkali degradation blank is prepared in the same way without using analyte. Under alkaline hydrolysis, percent recovery obtained for Tolvaptan was 92.251 % with two peaks of degradants [17]. Fig. 5 displays the typical chromatogram.

4.3. Degradation under oxidative condition

To 1 mL standard stock solution of drug (1000 µg/mL), 1 mL of 30% H_2O_2 was added and volume made to 10 mL with methanol to get 100 µg/mL solution. The solution was refluxed for 2 hr at 60^0C and 1 mL diluted to 10 mL with methanol to get 10µg/mL solution. The blank is prepared in the same way without using analyte. Under oxidative degradation, percent recovery obtained for Tolvaptan was 95.046 % with two peaks of degradant [17,18]. The representative chromatogram is shown in Fig. 6.

4.4. Degradation under dry heat

Dry heat study was performed by keeping drug sample in oven (110^0 C) for a period of 4 hour. A sample was withdrawn, 10 mg of it was dissolved in methanol to get solution of 1000 µg/mL and further diluted with methanol to get 10µg/mL as final concentration and was injected. Under dry heat degradation condition, percent recovery obtained for Tolvaptan was 99.618 % with no peak of degradant [18]. The representative chromatogram is shown in Fig.7.

4.5. Photo-degradation studies

By exposing the drug to UV light with illumination of NLT 200watt hr/m2 and exposure to cool white fluorescence light with illumination of NLT 1.2 million Lux-Hr, the photo degradation stability analysis of the drug was examined. Following exposure, 10 mg of the medication, precisely weighed, was placed to a volumetric flask with a capacity of 10 ml, which was then filled with methanol. Further diluted with methanol to a final concentration of 10mcg/ml, then injected. After exposure to UV light, an average of 99.174% of Tolvaptan was recovered with no peak of degradant, and after exposure to fluorescence light, an average of 99.451% of Tolvaptan was recovered with no peak of degradant. Figures 8 and 9 show the representative chromatogram, respectively [21,22,23].

5. Summary of degradation conditions

Summary of degradation study is in table no. 2.

6. Degradation Studies by LC-MS

Acid treated Tolvaptan degradant and its LC-MS spectra is given below (Fig. 12 & 13). Degradation pathway for degradants (D1, D2 & D3) is drawn in Fig. 14. The summery of degradation study parameters by acid, alkali and oxidation treatment and their RT with m/z ratio is given in table no. 3.

7. Conclusion

The proposed method quantitatively evaluated in terms of linearity, accuracy, precision, assay, robustness as per ICH Q2 R1 guideline. All these factors lead to the conclusion that the proposed method is simple, accurate, precise, and sensitive. Degradant's peak is well separated from analyte's peak so the proposed method can be used for the routine analysis of drug. Results from the method validation can be considered to judge the quality, reliability as well consistency of analytical results. The present study will be further extended for the study of degradants used as active ingredient and also for bioanalytical study.

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