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Development of a Stability Indicating UPLC Method for the Determination of Tirbanibulin in Bulk and its Pharmaceutical Dosage Form

Short Title: Stability Indicating UPLC Method for Tirbanibulin

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ABSTRACT
Objectives: The primary goal of this study was to create and validate a simple, precise, sensitive, and accurate UPLC method for estimating tirbanibulin in pure and dosage form.
Materials and Methods: The UPLC technique was developed using a Waters Acquity UPLC Phenyl (100x2.1mm,1.7m) column. The developed technique was validated in accordance with the International Conference on Harmonization's guidelines (ICH).
Results: Tirbanibulin was separated chromatographically with high resolutions using the mobile phase acetonitrile: buffer (30:70 v/v) at 0.5 mL/min, 5 µL injection volume, and 220 nm wavelength. The validated technique was found to be linear in the 1—15 µg/mL range. The detection and quantification limits for tirbanibulin were determined to be 0.03 and 0.1µ g/mL, respectively. The percentage RSD was less than 2%, demonstrating the precision of the developed technique. Furthermore, the recovery rate was nearly 100%, confirming the method's accuracy. Minor modifications to the chromatographic conditions demonstrated the method's robustness.
Conclusion: The developed analytical method was precise, simple, reproducible, and sensitive. As a result, it can be used to determine tirbanibulin.

Key words: UPLC, tirbanibulin, apoptosis, actinic keratosis, forced degradation studies

INTRODUCTION

Actinic keratosis (AK) is a precancerous skin condition affecting the face, balding scalp, and extremities. It is caused by the proliferation of atypical keratinocytes in response to prolonged intermittent UV light exposure. AK starts with DNA damage and mutation, then progresses to neoplastic transformation and growth [1]. These lesions may develop into squamous cell carcinoma (SCC) once abnormal cell invasion affects the dermis structures and has the potential to metastasize [2]. Long-term UV exposure causes a variety of epigenetic and

genetic alterations, disrupting the activity of crucial genes in keratinocytes that promote the progression of AK to SCC [3].
The chemical name of tirbanibulin is N-benzyl-2-(5-(4-(2-morpholinoethoxy) phenyl) pyridine-2-yal) acetamide. Tirbanibulin is a microtubule inhibitor. Tirbanibulin is a non-ATP-competitive inhibitor that also disrupts the proto-oncogenic Src tyrosine kinase signalling pathway [4]. Tirbanibulin also promotes the G2/M arrest of proliferating cell populations, upregulates p53, and triggers apoptosis by activating caspase-3 and cleaving poly (ADP-ribose) polymerase. Only topical use of tirbanibulin is currently approved. Tibranibulin should not be applied in close proximity to the mouth, lips, as well as eyes. Patients must take extra care to avoid getting tirbanibulin in their eyes or periocular region because it can cause unfavourable ophthalmic reactions. At the moment, only AKs on the face and scalp can be treated with tirbanibulin [5].
A thorough review of the literature revealed that there was no reported ultra-performance liquid chromatography method for estimating tirbanibulin that could indicate stability. Due to the high cost and fragility of analytical studies conducted using GC/MS or LC-MS in comparison to ultra-performance liquid chromatography (UPLC), the main focus was on developing an analytical method that was quick, precise, repeatable, and affordable. UPLC method was chosen for the developing stability-indicating method for determining tirbanibulin. According to the Q2 (R1) guidelines of the International Conference on Harmonization (ICH) procedure, the developed method was validated [6]. In this experiment, a UPLC method for the determination of the concentration of tirbanibulin in bulk form was established. The method was further successfully applied to the determination of tirbanibulin in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Tirbanibulin (Figure 1) pure bulk drug, tirbanibulin marketed dosage form (Klysiri), orthophosphoric acid, acetonitrile (HPLC grade), HPLC grade Water (Milli Q or equivalent) were the chemical materials and reagents used. All HPLC grade solvents were produced by Merck Company (Mumbai, India). The tirbanibulin drug sample was obtained from Shree Icon labs, Vijayawada, Andhra Pradesh, India as gift sample.

Instrument and chromatographic conditions

Chromatographic analysis was accomplished by means of a Waters Acquity ultra performance liquid chromatographic system equipped with PDA detector. A Phenyl chromatographic column (100x2.1mm,1.7µm) was employed with a flow rate of 0.5 mL/min. The following additional parameters were also set: an injection volume of 5.0 µL, and ambient column temperature. Acetonitrile and buffer were used in the mobile phase in a 30:70 ratio. There was a 2.0-minute runtime.

Preparation of buffer

Orthophosphoric acid of volume 1 mL was taken and dissolved in 1 liter of HPLC grade water.

Preparation of mobile phase

Acetonitrile and buffer were mixed in the ratio of 30:70 and filtered through 0.45µm membrane filter paper.

Preparation of standard stock solution

Five milligrams of tirbanibulin was weighed and transferred to a 50 mL volumetric flask. It was then diluted to volume with acetonitrile (diluent) to prepare a standard solution.

Preparation of standard working solution

Five millilitres (5 mL) of the standard stock solution were pipetted into a volumetric flask (50 mL). It was then reconstituted using the diluent (acetonitrile).

Preparation of sample stock solution

Tirbanibulin sample of 100 mg was accurately weighed and transferred into a 10mL volumetric flask by adding some diluent (acetonitrile). Then, it was sonicated to dissolve for 15 min and diluted to volume with diluent.

Preparation of sample working solution

From the sample standard solution, 1 mL was pipetted out and transferred into a volumetric flask (10 mL). Then, it was made up with diluent (acetonitrile).

Method validation
The analytical method validation for the estimation of assay of tirbanibulin in bulk and pharmaceutical dosage form was conducted. According to ICH guidelines, the effectiveness of analytical method validation parameters such as system suitability, linearity, limit of detection (LOD), limit of quantitation (LOQ), specificity (interference and forced degradation), precision (method precision and intermediate precision), robustness and accuracy was done to show the method's suitability for the estimation of tirbanibulin assay.

System suitability
By injecting 5 µL of the sensitivity standard solution in 6 replicates, the system's suitability was demonstrated. Standard chromatograms were used to assess the system suitability parameters such as plate count, relative standard deviations, retention time, resolution and variation.

Selectivity
A comparison-based approach was employed for ensuring the absence of interference and confirming method selectivity. Chromatograms of blank samples and placebo samples were used for this purpose.

Linearity
Tirbanibulin concentration and peak area were plotted to assess the analytical method's linearity and demonstrate that the absorbance is directly proportional to the analyte concentration in the sample. Tirbanibulin stock solution (100 µg/mL) was serially diluted to concentrations ranging from 1.0 to 15 µg/mL and then tested in accordance with a test procedure. A regression line was used to calculate the correlation coefficient (CC), regression coefficient (R^2), intercept, and slope.

Precision
To obtain method and intermediate precision, samples at 10 µg/ml concentration level were analysed and on each validation day, individual calibrations were carried out. Six different test preparations with a strength of 10 µg/mL each were assayed in order to evaluate the test method's precision. For intermediate precision, evaluation was completed by carrying out the required assay for six different test preparations with a strength of 10 µg/mL. Relative standard deviation (RSD%) was used to express precision.

Accuracy
For tirbanibulin, a recovery study was conducted at concentrations between 50% and 150% of the initial assay value. Individual level sample solutions were made in triplicate, and test-specific method analyses were conducted. The percentages of recovery, mean recovery, and RSD were computed.

Sensitivity
The lower limit of quantification and the limit of detection were derived by means of the subsequent equations based upon the slope of the calibration curve and the SD of responses.
LOD = 3.3 × Standard deviation (SD) / slope
LOQ = 10 × Standard deviation (SD) / slope

Assay of tirbanibulin
Sample (100 mg) was accurately weighed and transferred into a 10mL volumetric flask by adding some diluent. Then, it was sonicated to dissolve for 15 min and diluted to volume with diluent. From the sample standard solution, 1 mL was pipetted out and transferred into a volumetric flask (10 mL). Then, it was made up with diluent.

Robustness
By purposefully altering chromatographic parameters, the robustness of the analytical technique was examined under a range of circumstances. The impact of the acetonitrile and buffer composition in organic phase plus (33:67) and organic phase minus (27:73), as well as impact of flow rate plus (0.55 mL/min) and flow rate minus (0.45 mL/min) were assessed during the robustness study. In all the robustness conditions, the theoretical plates, %RSD and tailing factor were evaluated as system suitability criteria. Each robustness condition received an injection of a spiked sample, and the resolution between impurities was assessed.
Forced degradation studies
A study was conducted to show the effective separation of degradants from tirbanibulin peak in assay method.

Acid degradation
Accurately weighed 1 mL of sample stock solution was transferred to a dry and clean 10 mL volumetric flask.

To this solution, 1 millilitre of 1N hydrochloric acid was added & left undisturbed for fifteen minutes. Then, 1 mL of 1N NaOH was added to neutralize and made up to the mark with diluent (acetonitrile). Samples were taken out and processed through an UPLC at certain intervals of time (0, 6, 12, 18, and 24 hours).

Base degradation
The possible degradation peaks and rate of degradation of tirbanibulin were assessed by weighing sample stock solution of 1 mL and transferring into a ten millilitres volumetric flask. Then, it was subjected to forced degradation by adding 1mL of 1N NaOH and left for fifteen minutes. After fifteen minutes, 1 mL of 1N HCl was added and left. Then, diluted with diluent (acetonitrile) to volume. Samples were withdrawn at specific time (0, 6, 12, 18, and 24 hrs) intervals and subjected to UPLC runs.

Hydrolytic degradation
Tirbanibulin was tested for rate of degradation and potential degradation peaks. 1 mL of the sample stock solution was transferred to a volumetric flask with a volume of 10 mL. Then, it was forcedly degraded by adding 3 mL of HPLC grade water and kept aside for fifteen minutes. Then, sample was diluted with diluent to volume. Further, 1 mL was pipetted out into a 10 mL volumetric flask and made up with diluent (acetonitrile) up to the mark. Samples were taken out and processed through an UPLC at certain time points (0, 6, 12, 18, and 24 hours).

Peroxide degradation
Peroxide degradation was performed by assessing the probable peaks and rate of degradation of tirbanibulin by weighing sample stock solution of 1 mL. Then, the solution was transferred into a volumetric flask of volume 10 ml. Further, it was subjected to forced degradation by adding 1mL of 10% H_2O_2. It was left for fifteen minutes. Then, sample was diluted to volume with diluent (acetonitrile) and mixed. Further, 1 mL was pipetted out and diluted to 10 mL with diluent. Samples were withdrawn at specific time (0, 6, 12, 18, and 24 hrs) intervals and subjected to UPLC runs.
Reduction degradation
Tirbanibulin was tested for rate of degradation and potential degradation peaks. A volume of one millilitre of the sample stock solution was transferred into a ten millilitres volumetric flask. Then, it was forcedly degraded by adding 1 mL of 10 % sodium bi sulphate. It was undisturbed for fifteen minutes. Then, sample was diluted with diluent (acetonitrile) to volume. Samples were taken out and processed through an UPLC at certain intervals of time (0, 6, 12, 18, and 24 hours).

Thermal degradation
Sample of 500 mg was weighed and exposed at 105°C for 6 hrs. The exposed sample was used for analysis. The sample (100 mg) was transferred into a volumetric flask (10 mL). This sample was diluted with diluent and sonicated for 15 minutes to solubilize the contents. Further, 1 mL was pipetted into a 10 mL volumetric flask and diluted with diluent (acetonitrile). Samples at specific time (0, 6, 12, 18, and 24 hrs) points were withdrawn and put through UPLC runs to identify probable degradation chromatograms.

Photo stability degradation
The degradation rate and possible peaks of degradation for tirbanibulin were assessed by weighing 500 mg of sample. The sample was exposed for 6 hours at sunlight. 100 mg of the above sample was weighed. Then, transferred to a volumetric flask (10 mL). Diluents were added to this and sonicated for 15 minutes to dissolve the contents. Further, from the above solution 1 mL was pipetted out into a volumetric flask of volume 10 millilitres and made up to volume with diluent (acetonitrile). Samples at specific time (0, 6, 12, 18, and 24 hrs) points were withdrawn and put through UPLC runs.

RESULTS
Optimized process
After a series of trials, the mobile phase composition of acetonitrile: buffer in the proportion of 30:70 (v/v) had shown both peaks with good resolution, tailing factor, theoretical plate count. Hence this method was optimized and validated. Waters Acquity LC auto sampler enabled the elution, method development and validation of tirbanibulin. The method was proven to be simple to use, with high recovery, high sensitivity, and high specificity through thorough methodological validation. The optimized chromatogram was shown in Fig. 2.

System suitability parameters

Many analytical processes include testing for system compatibility. The system suitability characteristics were investigated and used to determine the best settings. The theoretical plate number (N) retention time, and tailing factor (T) were all studied for this purpose. Six replicate injections of the tirbanibulin standard working solution were used to carry out the procedure. It was observed that tirbanibulin retained for 0.814(average) min, with a tailing factor of not more than 1.31 in all peaks, indicating good peak symmetry. Theoretical plates were discovered to be greater than 3981 in all peaks. The results were tabulated in Table 1.

Linearity
The analytical method's linearity was its capacity to produce test results within a specified range that were directly proportional to the concentration of the analyte in the test sample. Seven concentration levels in the range of 1 and 15 µg/mL were used to test the analytical method's linearity. The regression coefficient, y-intercept, and slope of regression line were calculated. The observed corelation coefficient value was 0.9996. The results were shown in Tables 2-3 and Figure 3.

Accuracy
A method's accuracy reflects how closely the outcomes it produces match the actual value. According to the accuracy results, % RSD was 0.11%, and percentage recovery at all three levels ranged from 99.7 to 100.9%. The results were tabulated in Table 4.

Precision
When multiple samples of the same homogeneous sample were taken under the specified conditions, the precision of the method was defined as the degree of agreement between the measurements obtained. Relative standard deviation was typically used to express precision. The percent relative standard deviation value for method precision results of tirbanibulin was found to be 0.47%. The percent relative standard deviation value for intermediate precision results of tirbanibulin was found to be 0.33%. The results were showed in Tables 5-6.

Sensitivity
The limit of quantification (LOQ) was defined as the lowest amount of analyte in a sample that can be quantitatively determined with appropriate precision, as opposed to the limit of detection (LOD), which was the lowest amount of analyte in a sample that can be detected but not necessarily quantitated. The LOD and LOQ for tirbanibulin were 0.03 and 0.1 µg/mL respectively. The results were tabulated in Table 7.

Assay
According to the label claim, the drug content obtained from the values of sample solutions was found to be in the permissible range of 90–110 percent. The % assay of tirbanibulin was found to be 100.5% w/w. The results were displayed in Table 8.

Robustness
The influence of slight alterations in chromatographic settings was used to determine the robustness of the analytical process. The percent RSD of tirbanibulin was less than 2.0 in all of the deliberately changed chromatographic settings. The results were showed in the Table 9.

Selectivity
In the retention time ranges, the UPLC chromatograms for the drug matrix (combination of the medicine and placebos) revealed nearly no interference peaks. As a result, the proposed UPLC approach in this study was selective. The method's specificity and selectivity were tested by looking for interference peaks in the chromatograms of blank and placebo samples. Because of the excipients, there were no interfering peaks. As a result, the procedure was specific and selective. Figures 4 and 5 show the chromatograms of blank and working placebo solution respectively.

Forced degradation studies
Tirbanibulin was subjected to a variety of stress conditions, which include hydrolysis, base, oxidative, acid, photo stability, and thermal degradation, as per ICH guidelines. The proposed UPLC approach was used to monitor degradation behaviour on a regular basis. The PDA detector results from the forced deterioration results revealed that the tirbanibulin peaks were pure and homogenous in all of the stressful conditions studied. This demonstrates that the approach is both particular and stable. All the results of stability studies were displayed in Tables 10 - 16. The degradation chromatograms were displayed in Figures 6- 17.

DISCUSSION

Tirbanibulin is a microtubule inhibitor. Tirbanibulin is a non-ATP-competitive inhibitor that also disrupts the proto-oncogenic Src tyrosine kinase signalling pathway [4]. Therefore, a technique for tirbanibulin determination is required. According to the ICH, a system suitability test is frequently used to assess a chromatographic system's resolution, column efficiency, and repeatability to make sure it is suitable for a certain analysis [6]. The new approach was tuned to produce a symmetrical peak and high theoretical plates (N). The total number of theoretical plates was above 2000, which was deemed sufficient for the system suitability test. According to the standards, the tailing factor was within the specified limitations. These findings demonstrate that the proposed strategy can produce data of acceptable quality. The suggested method's application to the analysis of formulations is its key feature. Hence, the market sample of tirbanibulin was collected and analysed by employing the proposed method. The study confirmed that the created UPLC method was accurate and easy enough to be used on a daily basis. The suggested assay method's high content results indicate that this technique can be engaged for quantitative regular quality control study of pharmaceutical dosage forms. The analytical method's linearity was its capacity to produce test findings within a specified range that directly relate to the analyte concentration in the test sample [6]. The regression line of analysis expresses the relationship between concentration and peak area of tirbanibulin. As a result, the findings revealed that the peak area and analyte concentration showed a strong correlation. The R^2 high value indicated good linearity. An analytical technique's accuracy shows the closeness of outcomes produced by that technique with the true value [6]. The percentage recovery and percent RSD results fell within the acceptable ranges of 98.0% to 102.0% and not more than 2.0%, respectively, demonstrating the method's suitability for routine drug analysis. The degree of agreement between a set of measurements made using repeated samples of the same homogeneous material under the specified conditions was considered the precision of the method, and it was typically stated as relative standard deviation[6]. The results were well under the usually accepted 2 percent limit. As a result, the new method's precision has been confirmed. The limit of quantification (LOQ) was defined as the smallest amount of analyte in a sample that could be identified quantitatively with adequate accuracy. The lowermost amount of analyte in a sample that could be detected but not necessarily quantitated was defined as the limit of detection (LOD) [6]. The LOD and LOQ for tirbanibulin were observed to be 0.03 and 0.1 µg/mL respectively. The system suitability parameters were not significantly changed while varying the conditions, hence the method was robust. In the retention time ranges, the UPLC chromatograms for the drug matrix (combination of the medicine and placebos) revealed nearly no interference peaks. As a result, the proposed UPLC approach in this study was selective. The drug tirbanibulin was found to undergo extreme degradation in peroxide degradation conditions. The tirbanibulin peaks were homogenous and showed results within acceptance limits in all the forced degradation studies. This demonstrates that the approach is both particular and stable. The developed method effectively passed all the validation parameters and was applied efficaciously for the determination of tirbanibulin.

CONCLUSION

For the estimate of tirbanibulin in dosage form, a simple, accurate, and specific approach was established. Tirbanibulin had retention time of 0.811 minutes. The percent RSD of method precision and intermediate precision was found to be 0.47 and 0.33 percentage. For tirbanibulin, percent recovery was 100.1 percent. The LOD and LOQ values for tirbanibulin calculated from regression equations were 0.03 µg/mL and 0.1 µg/mL consecutively. Regression equation of tirbanibulin was y = 309884.02x +29801.60. There were some degradation peaks in acid, base, thermal, reduction and peroxide stressed conditions according to the results of the forced degradation test. Because retention times and run times were reduced, the method created was simple and economic, and it might be utilized in frequent quality control tests in industries.

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Informed Consent: Not applicable.
Authorship Contributions
Concept: G.P.K., S.R., Design: G.P.K., S.R., Data Collection or Processing: G.P.K., Analysis or Interpretation: G.P.K., Literature Search: G.P.K., Writing: G.P.K.

CONFLICT OF INTEREST

The authors declare that there is no conflict of competing financial interests.

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TABLES

Table 1 System suitability parameters for tirbanibulin

S. No	RT (min)	USP Plate Count	Tailing
1	0.811	4004	1.31
2	0.814	3981	1.31
3	0.810	4008	1.31
4	0.818	4067	1.27
5	0.818	4016	1.30
6	0.817	4022	1.30

Table 2. Linearity studies of tirbanibulin

S. No	Concentration (µg/mL)	Peak area
1	1	352095
2	2.5	824524
3	5	1578906
4	7.5	2411569
5	10	3039528
6	12.5	3919186
7	15	4691400

Table 3. Optical characteristics of tirbanibulin

Parameters	Tirbanibulin
Linearity (µg/mL)	1-15 µg/mL
Regression equation	y = 309884.02x +29801.60
Slope	309884.02
Intercept	29801.60
Correlation coefficient (R^2)	0.9996

Table 4. Recovery studies of tirbanibulin

Recovery level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean	SD	% RSD

50%	0.50	0.497	99.4	100	0.60	0.6
	0.50	0.5	100			
	0.50	0.503	100.6			
100%	1.0	1.001	100.1	100.2	0.50	0.5
	1.0	0.997	99.7			
	1.0	1.007	100.7			
150%	1.5	1.495	99.7	100.2	0.61	0.6
	1.5	1.502	100.1			
	1.5	1.513	100.9			
Mean				100.1		
SD				0.115		
% RSD				0.11		

*SD: standard deviation, RSD: relative standard deviation

Table 5. Method precision studies of tirbanibulin

S. No	Area of tirbanibulin	% Label claim
1.	3024698	100
2.	3046952	100.7
3.	3036756	100.4
4.	3065289	101.3
5.	3026985	100.1
6.	3034356	100.3
Mean		100.5
S. D		0.476
%RSD		0.47

*SD: standard deviation, RSD: relative standard deviation

Table 6. Intermediate precision studies of tirbanibulin

S. No	Area of tirbanibulin	% Label claim
1.	3026985	100.1
2.	3032534	100.3
3.	3042589	100.7
4.	3045824	100.8
5.	3049269	100.8
6.	3050687	100.9
Mean		100.6
S. D		0.337

%RSD	0.33

*SD: standard deviation, RSD: relative standard deviation

Table 7. LOD and LOQ of tirbanibulin

Drug	LOD (µg/mL)	LOQ (µg/mL)
Tirbanibulin	0.03	0.1

Table 8 Results of marketed formulation analysis.

Compound name	Brand name	Label claim (mg)	% Assay (%w/w)
Tirbanibulin	Klysiri	2.5	100.5

Table 9. Robustness studies of tirbanibulin

Condition	Peak area	% Label claim	Mean	S. D	% RSD
Flow rate (+) 0.55 ml/min	2785496	100.4	99.6	0.764	0.77
	2758463	99.4			
	2745818	98.9			
Flow rate (-) 0.45 ml/min	3365289	100.2	100.1	0.173	0.17
	3352144	99.9			
	3362569	100.2			
Mobile phase (+) 33 O:67 B	2652542	99.4	99.7	0.252	0.25
	2665324	99.9			
	2659868	99.7			
Mobile phase (-) 27 O:73 B	3345781	99.7	99.9	0.153	0.15
	3354787	99.9			
	3356253	100			

*SD: standard deviation, RSD: relative standard deviation

Table 10. Acid degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2896528	95.8	4.2	0.157	1.052
6 hrs	2701953	89.4	10.4	0.148	1.058
12 hrs	2542200	84.1	15.7	0.136	1.049

18 hrs	2416121	79.9	19.9	0.124	1.052
24 hrs	2318506	76.7	23.1	0.125	1.063

Table 11. Base degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2926358	96.8	3.2	0.163	1.047
6 hrs	2765472	91.5	8.5	0.166	1.047
12 hrs	2556387	84.6	15.4	0.139	1.037
18 hrs	2446953	81	19	0.149	1.071
24 hrs	2384105	78.9	21.1	0.129	1.034

Table 12. Peroxide degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2905874	96.1	3.9	0.144	1.057
6 hrs	2763592	91.4	8.6	0.142	1.053
12 hrs	2575219	85.2	14.8	0.159	1.066
18 hrs	2429856	80.4	19.6	0.162	1.062
24 hrs	2306598	76.3	23.7	0.168	1.069

Table 13. Reduction degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2910368	96.3	3.7	0.169	1.032
6 hrs	2758292	91.3	8.7	0.171	1.063
12 hrs	2552875	84.5	15.5	0.129	1.042
18 hrs	2455937	81.3	18.7	0.154	1.069
24 hrs	2340156	77.4	22.6	0.125	1.044

Table 14. Thermal degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2896237	95.8	4.2	0.135	1.064
6 hrs	2845421	94.1	5.9	0.129	1.072
12 hrs	2790124	92.3	7.7	0.145	1.076
18 hrs	2729638	90.3	9.7	0.138	1.068
24 hrs	2609856	86.3	13.7	0.147	1.064

Table 15. Photolysis degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2997541	99.2	0.8	0.178	1.074
6 hrs	2976412	98.5	1.5	0.149	1.069
12 hrs	2954638	97.8	2.2	0.143	1.052
18 hrs	2938721	97.2	2.8	0.146	1.054
24 hrs	2906352	96.2	3.8	0.158	1.069

Table 16. Hydrolytic degradation studies of tirbanibulin

Time	Peak area	% Label claim	% Degraded	Purity angle	Purity threshold
Initial	2980574	98.6	1.4	0.149	1.067
6 hrs	2967521	98.2	1.8	0.143	1.064
12 hrs	2932547	97	3	0.148	1.062
18 hrs	2916359	96.5	3.5	0.146	1.062
24 hrs	2902593	96	4	0.164	1.065

FIGURES

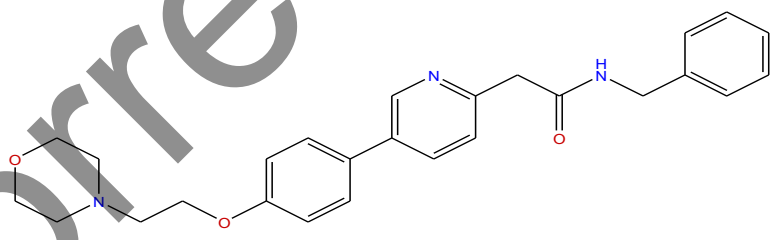

Figure 1. Structure of tirbanibulin

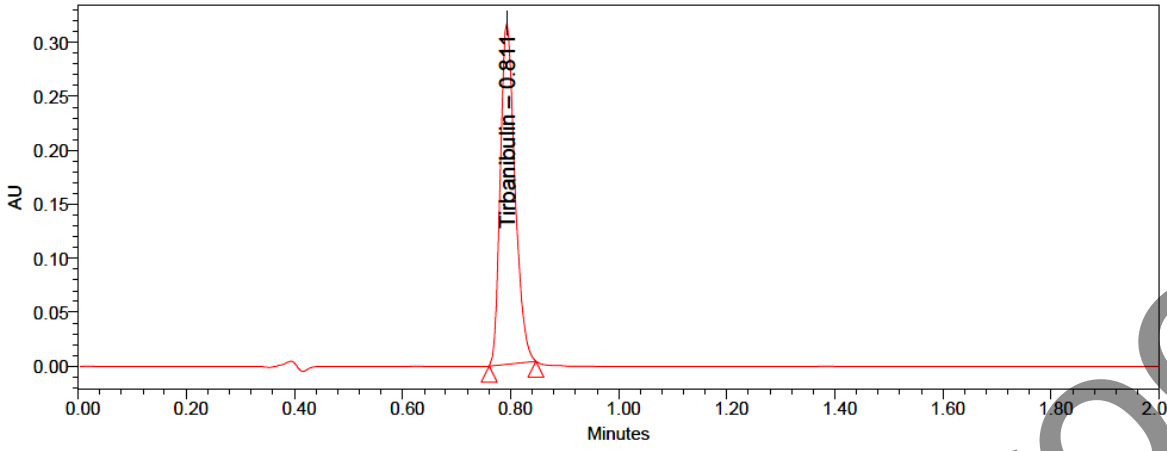

Figure 2. Optimized chromatogram of tirbanibulin

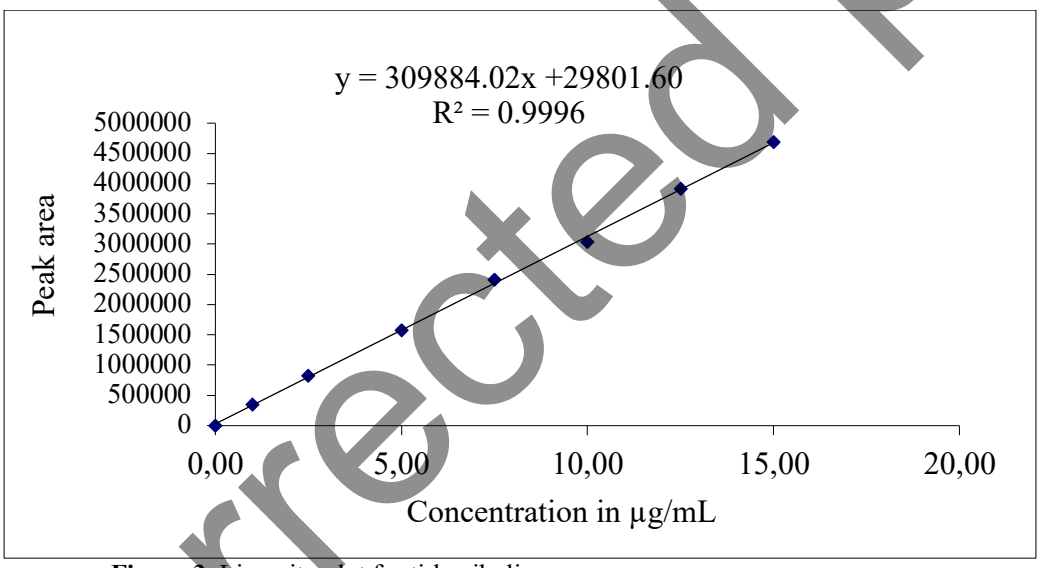

Figure 3. Linearity plot for tirbanibulin

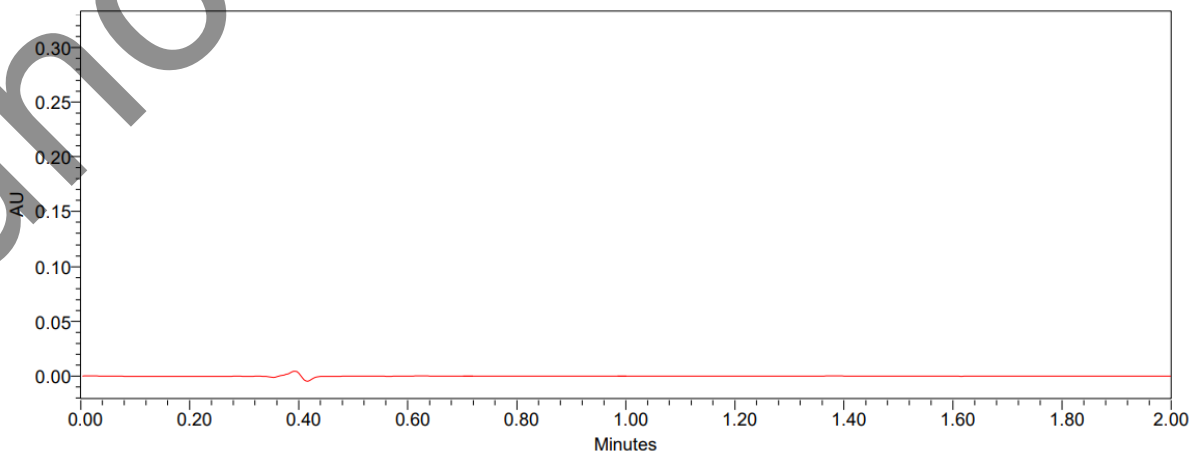

Figure 4. Chromatogram of blank

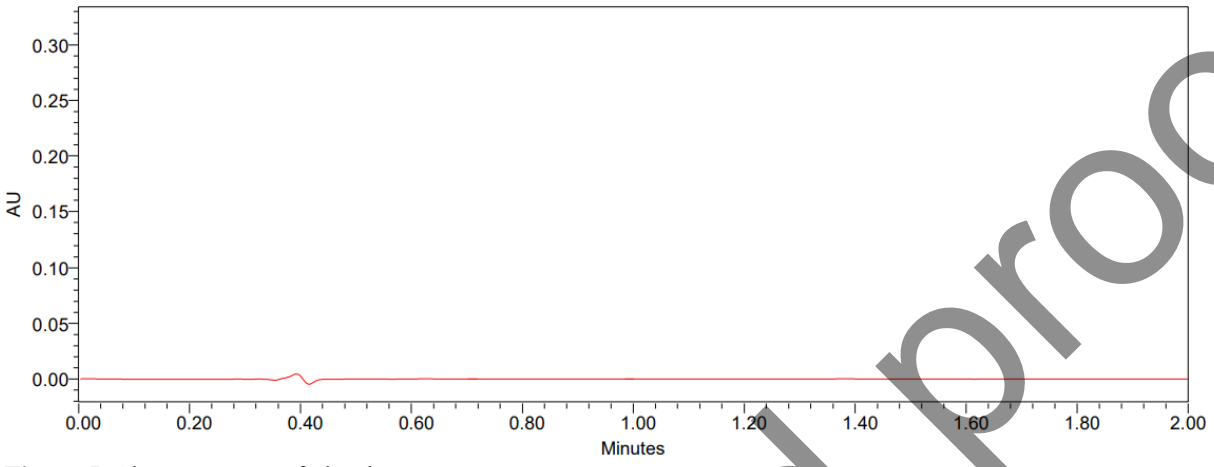

Figure 5. Chromatogram of placebo

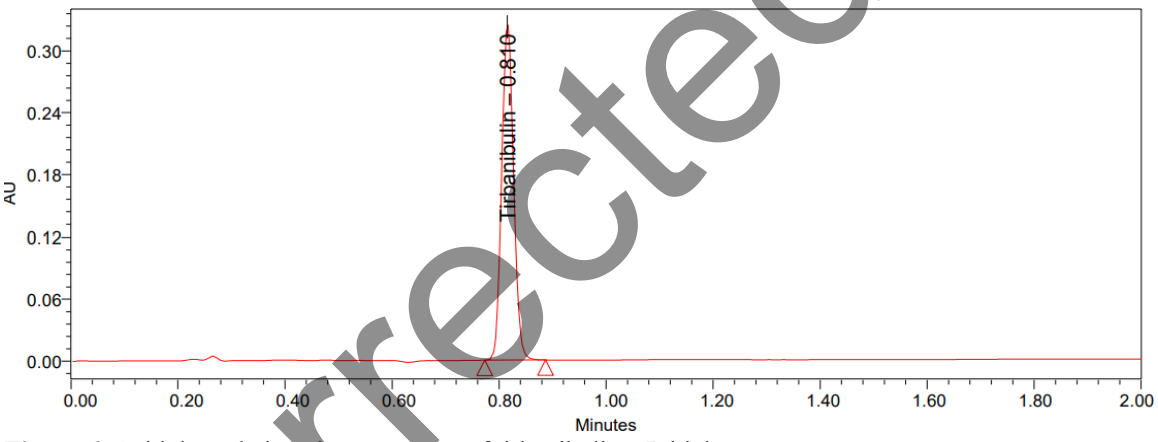

Figure 6. Acid degradation chromatogram of tirbanibulin – Initial

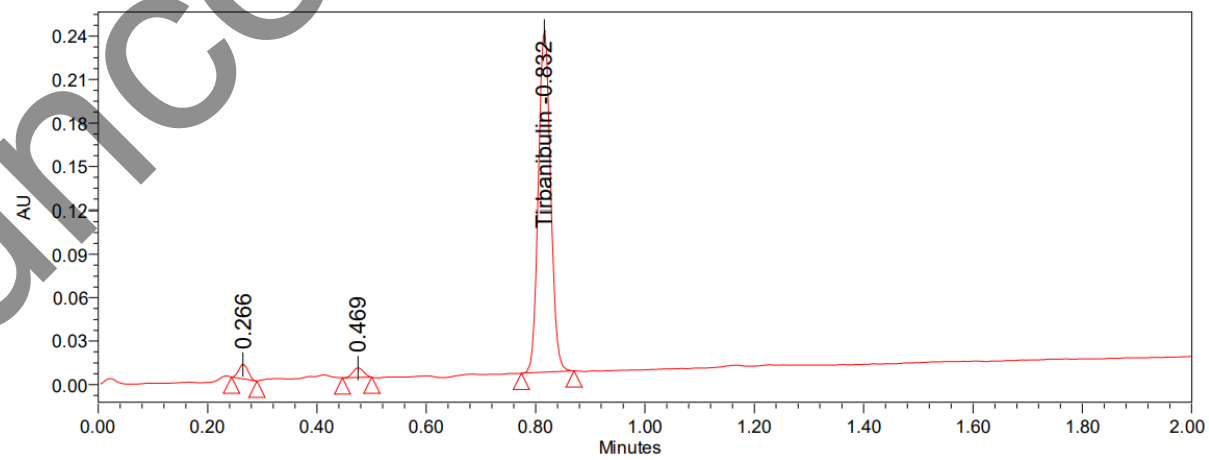

Figure 7. Acid degradation chromatogram of tirbanibulin – 24 h

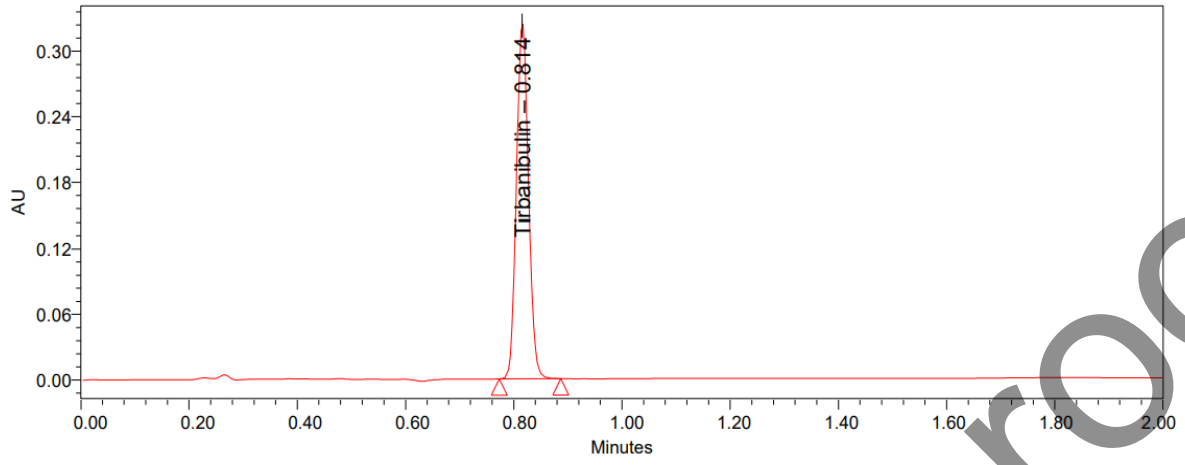

Figure 8. Base degradation chromatogram of tirbanibulin – Initial

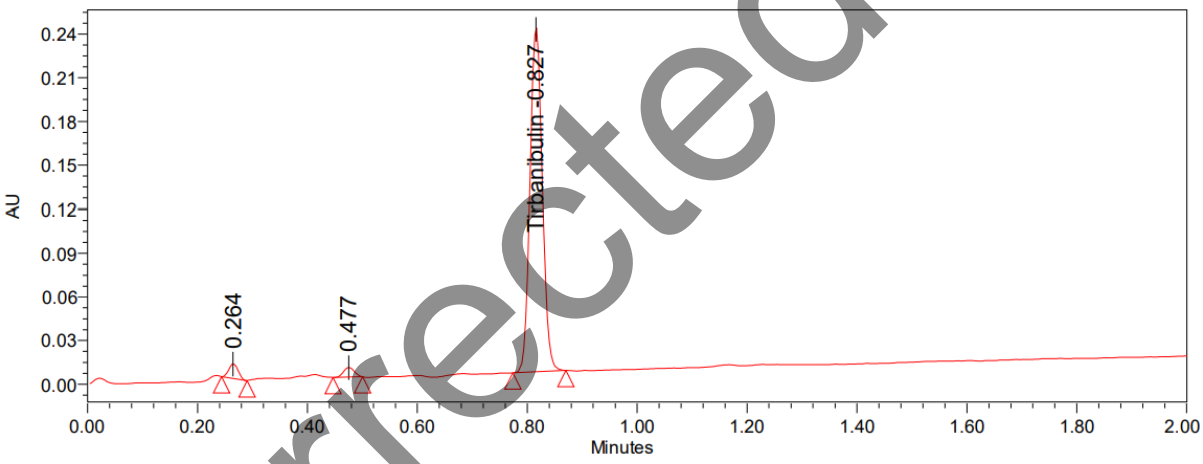

Figure 9. Base degradation chromatogram of tirbanibulin – 24 h

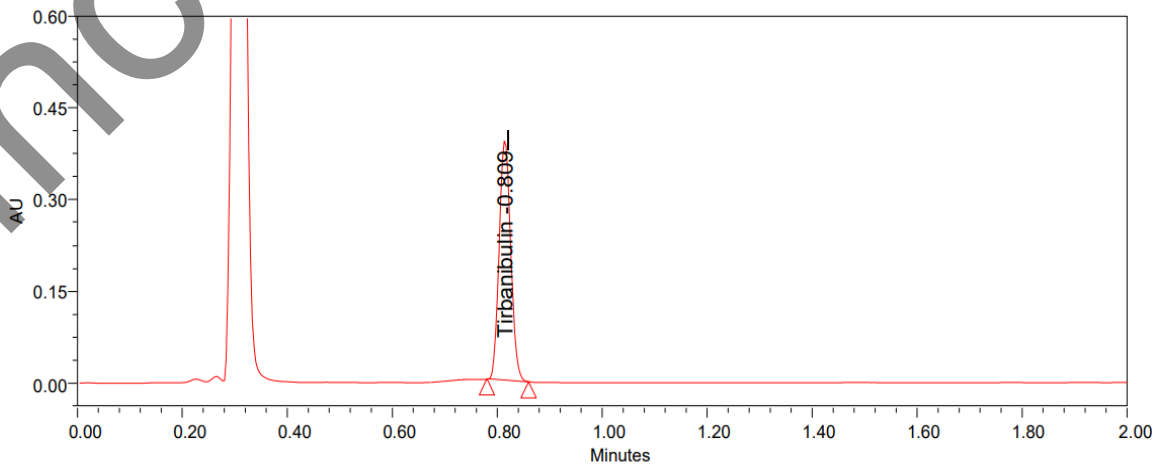

Figure 10. Peroxide degradation chromatogram of tirbanibulin - Initial

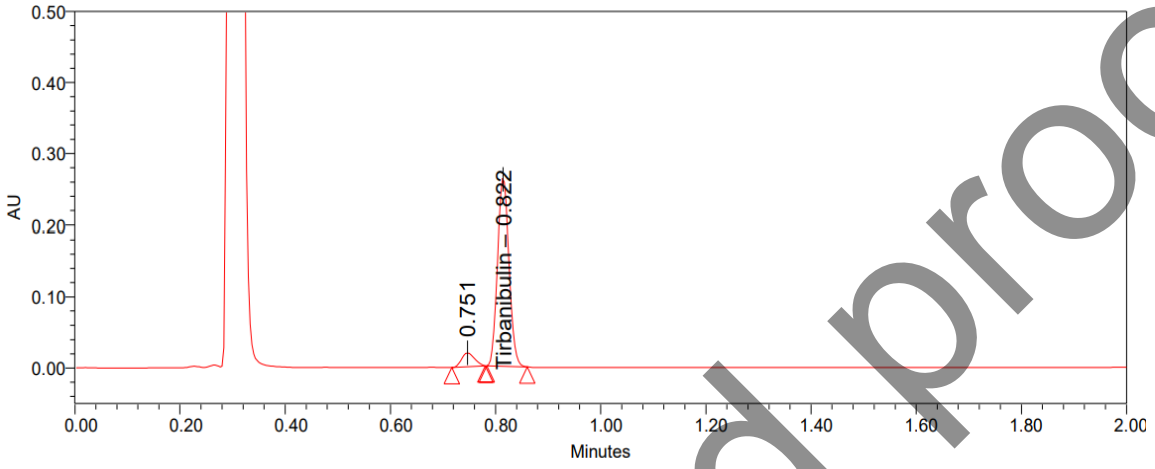

Figure 11. Peroxide degradation chromatogram of tirbanibulin – 24 h

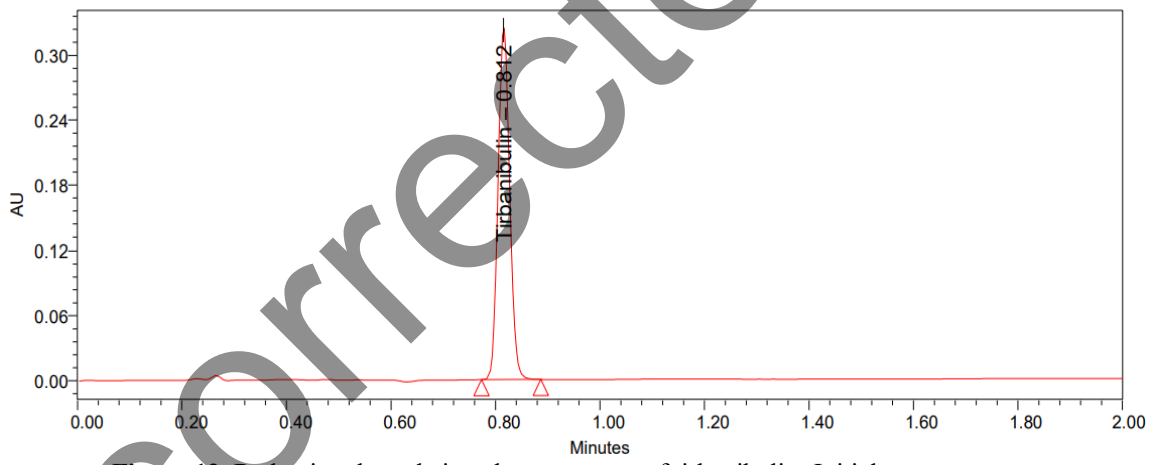

Figure 12. Reduction degradation chromatogram of tirbanibulin- Initial

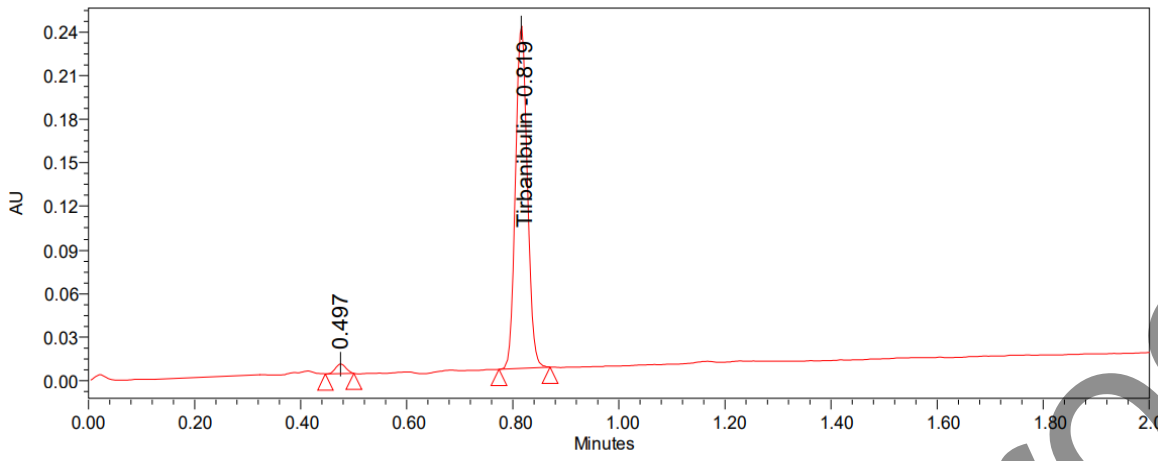

Figure 13. Reduction degradation chromatogram of tirbanibulin- 24h

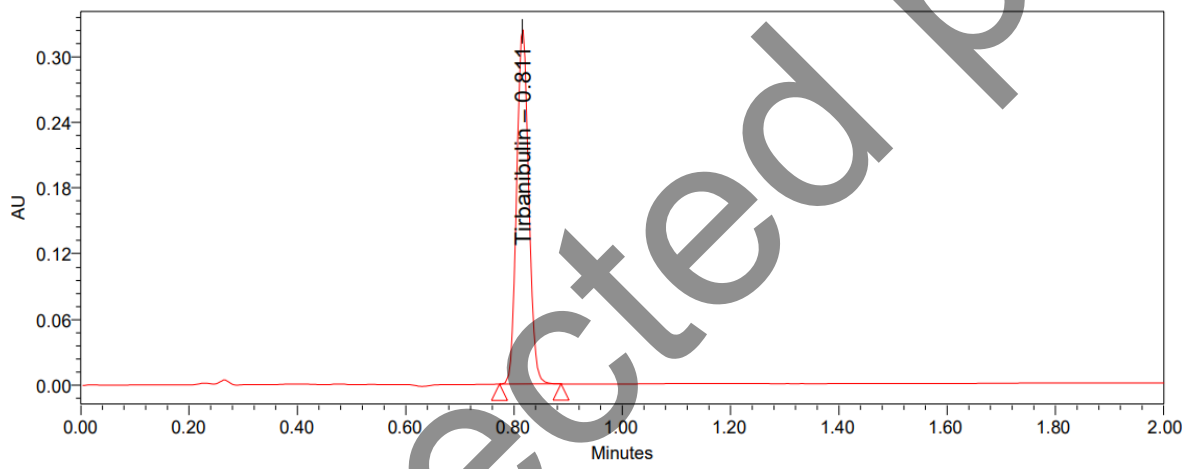

Figure 14. Thermal degradation chromatogram of tirbanibulin - Initial

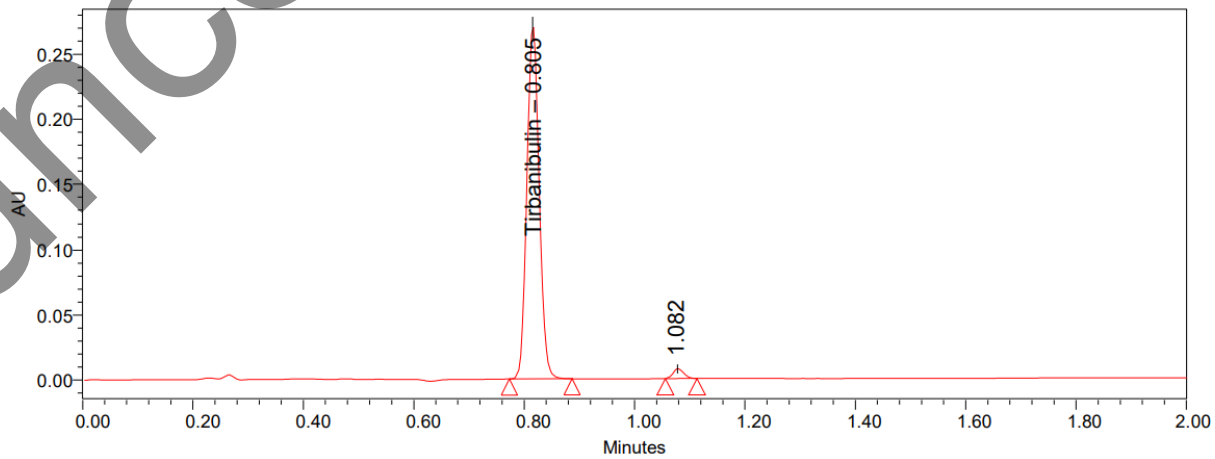

Figure 15. Thermal degradation chromatogram of tirbanibulin – 24 h

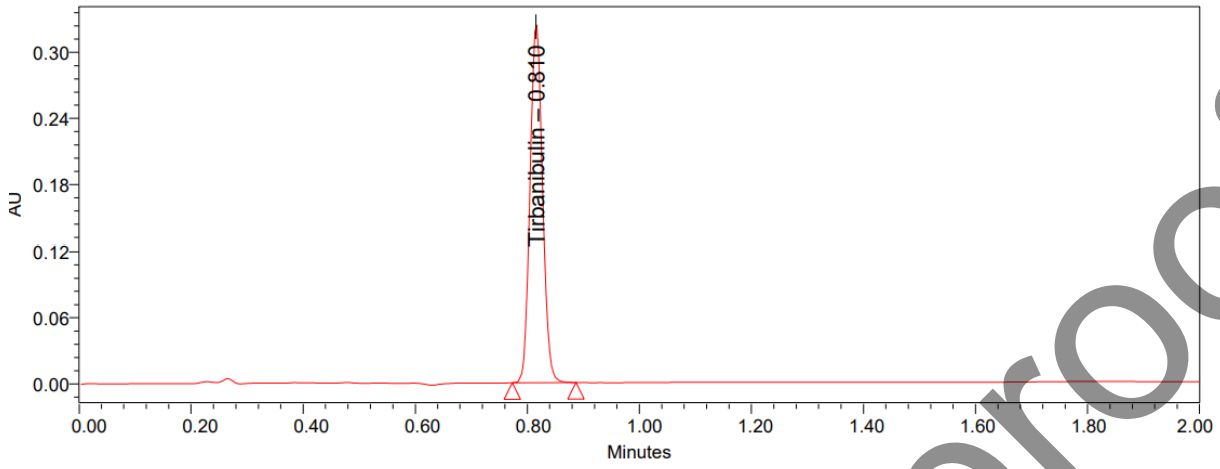

Figure 16. Hydrolysis degradation chromatogram of tirbanibulin – Initial

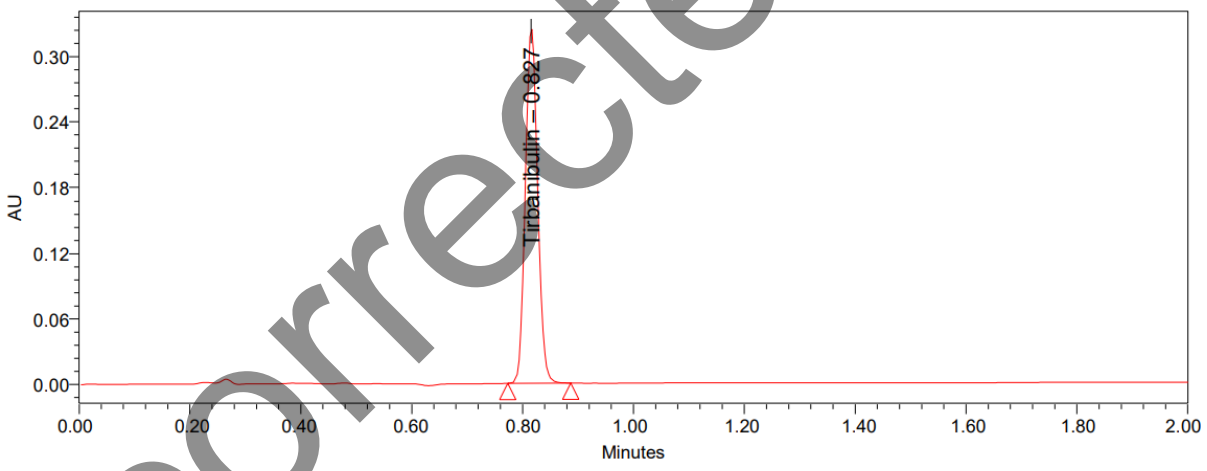

Figure 17. Hydrolysis degradation chromatogram of tirbanibulin – 24 h