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ORIGINAL ARTICLE

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

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ABSTRACT

Objectives: The primary goal of this study was to create and validate a simple, precise, sensitive, and accurate ultra-performance liquid chromatography (UPLC) method for estimating tirbanibulin in pure and dosage form.

Materials and Methods: A UPLC technique was developed using a Waters Acquity UPLC Phenyl (100 x 2.1 mm, 1.7 µm) column. The developed technique was validated in accordance with the International Conference on Harmonization (ICH) guidelines.

Results: Tirbanibulin was separated chromatographically with high resolution 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 0.03 and 0.1 µg/mL, respectively. The percentage relative standard deviation was less than 2%, demonstrating the precision of the developed technique. Furthermore, the recovery rate was nearly 100%, confirming the accuracy of the method. Minor modifications to the chromatographic conditions demonstrated the robustness of the method.

Conclusion: The developed analytical method was precise, simple, reproducible, and sensitive. Consequently, it can be used to determine tirbanibulin.

Keywords: 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 ultraviolet-visible (UV) light exposure. AK starts with DNA damage and mutation and 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 can metastasize.[2] Long-term UV exposure causes various 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-adenosine triphosphate (ATP)-competitive inhibitor that disrupts the proto-oncogenic Src tyrosine kinase signaling pathway.[4] Tirbanibulin also promotes the G2/M arrest of proliferating cell populations, upregulated *p53*, and triggers apoptosis by activating caspase-3 and cleaving poly [adenosine diphosphate (ADP) ribose] polymerase. Only the topical use of tirbanibulin is currently approved, which should not be applied in close proximity to the mouth, lips, or eyes. Patients must take extra care to avoid tirbanibulin in their eyes or periocular region because it can cause unfavorable ophthalmic reactions. At present, only AKs on the face and scalp can be treated with tirbanibulin.[5]

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A thorough review of the literature revealed that there is 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 gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (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. The UPLC method was chosen for the development of a 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 determining the concentration of tirbanibulin in bulk form was established. The method was further successfully applied to the determination of tirbanibulin in the pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and reagents

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

Instrument and chromatographic conditions

Chromatographic analysis was accomplished using a Waters Acquity ultra-performance liquid chromatographic system equipped with a PDA detector. A phenyl chromatographic column (100 x 2.1 mm, 1.7 µm) 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 at a 30:70 ratio. There was a 2.0-min runtime.

Preparation of the buffer

Orthophosphoric acid (1 mL) was taken and dissolved in 1 L of HPLC-grade water.

Preparation of the mobile phase

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

Preparation of the standard stock solution

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

Preparation of the standard working solution

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

Preparation of the sample stock solution

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

Preparation of the 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 a diluent (acetonitrile).

Method validation

The analytical method validation for the estimation of the assay of tirbanibulin in bulk and pharmaceutical dosage forms was conducted. According to the International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (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 evaluated 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 suitability of the system was demonstrated. Standard chromatograms were used to assess system suitability parameters such as plate count, relative standard deviations (RSDs), retention time, resolution, and variation.

Selectivity

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

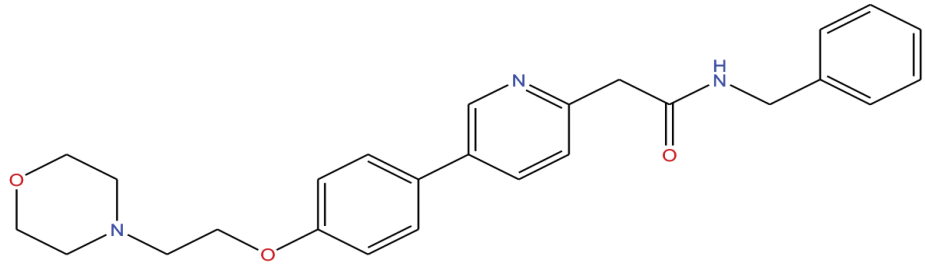

Figure 1. Structure of the tirbanibulin

Linearity

Tirbanibulin concentration and peak area were plotted to assess the linearity of the analytical method and demonstrate that the absorbance is directly proportional to the analyte concentration in the sample. The 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 the 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 analyzed, and on each validation day, individual calibrations were performed. Six different test preparations with a strength of 10 µg/mL each were assayed to evaluate the precision of the test method. For intermediate precision, evaluation was completed by performing the required assay for six different test preparations with a strength of 10 µg/mL. The 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 prepared in triplicate, and test-specific method analyses were conducted. Percentages of recovery, mean recovery, and RSD were computed.

Sensitivity

The lower LOQ and the LOD were derived using the subsequent equations based on the slope of the calibration curve and the SD of responses.

LOD = 3.3 × standard deviation (SD)/slope

LOQ = 10 × SD/slope

Assay of tirbanibulin

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

Robustness

By purposefully altering the 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 the 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 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 the tirbanibulin peak in the assay method.

Acid degradation

Accurately weighed 1 mL the sample stock solution was transferred to a dry and clean 10 mL volumetric flask. To this solution, 1 mL of 1 N hydrochloric acid was added and left undisturbed for 15 min. Then, 1 mL of 1 N NaOH was added to neutralize and made up to the mark with a diluent (acetonitrile). Samples were taken out and processed through a 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 a sample stock solution of 1 mL and transferring it into a 10 mL volumetric flask. Then, it was subjected to forced degradation by adding 1 mL of 1 N NaOH and left for 15 min. After 15 min, 1 mL of 1 N HCl was added and left. Then, it is diluted with diluent (acetonitrile) to volume. Samples were withdrawn at specific time (0, 6, 12, 18, and 24 hours) intervals and subjected to UPLC.

Hydrolytic degradation

Tirbanibulin was tested for the rate of degradation and potential degradation peaks. The sample stock solution (1 mL) 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 15 min. The 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 UPLC at certain time points (0, 6, 12, 18, and 24 hours).

Peroxide degradation

Peroxide degradation was performed by assessing the probable peaks and the rate of degradation of tirbanibulin by weighing a sample stock solution of 1 mL. Then, the solution was transferred into a volumetric flask of 10 mL. Further, it was subjected to forced degradation by adding 1 mL of 10% H_2O_2. It was left for fifteen minutes. Then, the sample was diluted to volume with diluent (acetonitrile) and mixed. Further, 1 mL was pipetted out and diluted to 10 mL with the diluent. Samples were withdrawn at a specific time (0, 6, 12, 18, and 24 hours) intervals and subjected to UPLC.

Reduction degradation

Tirbanibulin was tested for the rate of degradation and potential degradation peaks. A volume of 1 mL of the sample stock solution was transferred into a 10 mL volumetric flask. Then, it was forcedly degraded by adding 1 mL of 10% sodium bi-sulfate. It was undisturbed for 15 minutes. Then, the sample was diluted with diluent (acetonitrile) to volume. Samples were taken out and processed through a UPLC at certain intervals of time (0, 6, 12, 18, and 24 hours).

Thermal degradation

A sample of 500 mg was weighed and exposed at 105 °C for 6 hours. The exposed sample was used for the analysis. The sample (100 mg) was transferred into a volumetric flask (10 mL). This sample

was diluted with the diluent and sonicated for 15 min to solubilize the contents. Further, 1 mL was pipetted into a 10 mL volumetric flask and diluted with acetonitrile. Samples at a specific time (0, 6, 12, 18, and 24 hours) points were withdrawn and subjected to UPLC runs to identify probable degradation chromatograms.

Photostability degradation

The degradation rate and possible peaks of degradation for tirbanibulin were assessed by weighing 500 mg of the sample. The sample was exposed to sunlight for 6 hours. 100 mg of the above sample was weighed. Transfer to a volumetric flask (10 mL). Diluents were added and sonicated for 15 min to dissolve the contents. Further, from the above solution, 1 mL was pipetted out into a volumetric flask of volume 10 mL and made up to volume with diluent (acetonitrile). Samples at specific times (0, 6, 12, 18, and 24 hours) 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*) showed both peaks with good resolution, tailing factor, and theoretical plate count. Hence, this method was optimized and validated. The Waters Acquity LC autosampler 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 is shown in Figure 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 perform the procedure. It was observed that tirbanibulin was 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 are tabulated in Table 1.

Linearity

The linearity of the analytical method was its capacity to produce test results within a specified range that was directly proportional to the concentration of the analyte in the test sample. Seven concentrations in the range of 1 and 15 µg/mL were used to test the linearity of the analytical method. The regression coefficient, y-intercept, and slope of the regression line were calculated. The observed correlation coefficient value was 0.9996. The results are 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, the RSD (%) was 0.11%, and the percentage recovery at all three levels ranged from 99.7% to 100.9%. The results are tabulated in Table 4.

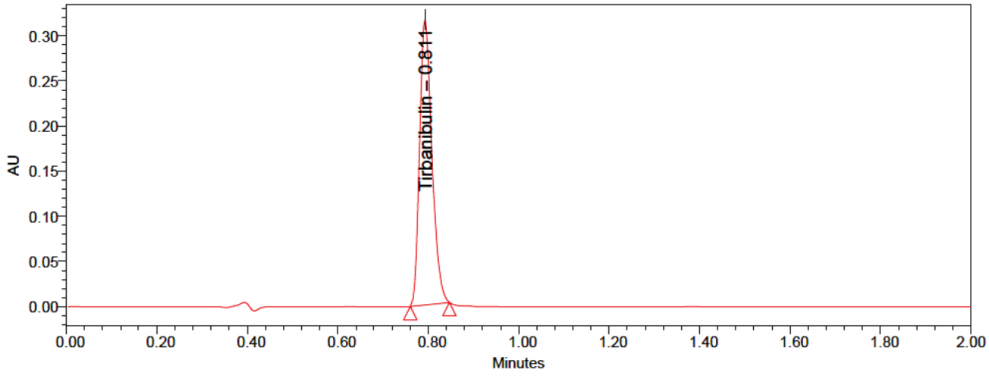

Figure 2. Optimized chromatogram of tirbanibulin
AU: Absorbance units

Table 1. System suitability parameters for tirbanibulin

Sample number	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

RT: Retention time, USP: United States Pharmacopoeia

Table 2. Linearity studies of tirbanibulin

Sample number	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

Precision

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

Sensitivity

The LOQ was defined as the lowest amount of analyte in a sample that could be quantitatively determined with appropriate precision, as opposed to the LOD, which was the lowest amount of analyte in a sample that could be detected but not necessarily

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

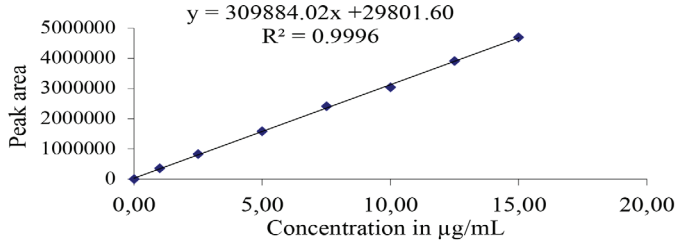

Figure 3. Linearity plot of tirbanibulin

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

Sample number	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
SD		0.476
RSD (%)		0.47

SD: Standard deviation, RSD: Relative standard deviation

Table 6. Intermediate precision studies of tirbanibulin

Sample number	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
SD		0.337
RSD (%)		0.33

SD: Standard deviation, RSD: Relative standard deviation

quantitated. The LOD and LOQ for tirbanibulin were 0.03 and 0.1 µg/mL, respectively. The results are tabulated in Table 7.

Assay

According to the label claim, the drug content obtained from the values of the sample solutions was found to be in the permissible range of 90-110%. The percentage assay of tirbanibulin was found to be 100.5% (*w/w*). The results are displayed in Table 8.

Robustness

The influence of slight alterations in the chromatographic settings was used to determine the robustness of the analytical process. The percent RSD of tirbanibulin was less than 2.0 in all deliberately changed chromatographic settings. The results are shown in 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. Consequently, the procedure was specific and selective. Figures 4 and 5 show the chromatograms of the blank and working placebo solutions, respectively.

Table 7. LOD and LOQ of tirbanibulin

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

LOD: Limit of detection, LOQ: Limit of quantification

Table 8. Results of the 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	SD	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

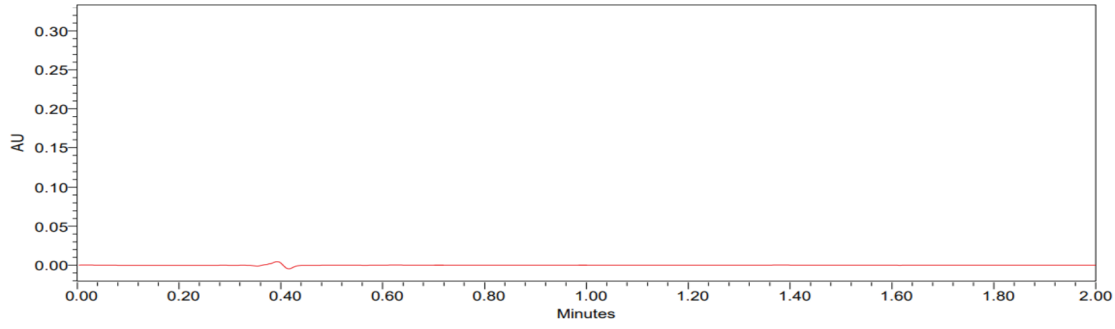

Figure 4. Chromatogram of the blank
AU: Absorbance units

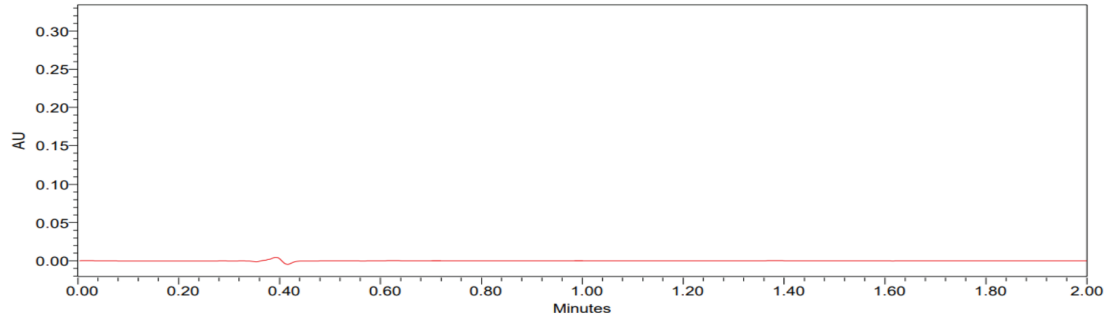

Figure 5. Chromatogram of the placebo
AU: Absorbance units

Forced degradation studies

Tirbanibulin was subjected to various stress conditions, including hydrolysis, base, oxidative, acid, photostability, and thermal degradation, as *per* the ICH guidelines. The proposed UPLC approach was used to monitor the degradation behavior regularly. 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 proposed approach is both particular and stable. All the results of the stability studies are displayed in Tables 10-16. The degradation chromatograms are displayed in Figures 6-17.

Table 10. Acid degradation of tirbanibulin

Time	Peak area	Label claim (%)	Degraded (%)	Purity angle	Purity threshold
Initial	2896528	95.8	4.2	0.157	1.052
6 hours	2701953	89.4	10.4	0.148	1.058
12 hours	2542200	84.1	15.7	0.136	1.049
18 hours	2416121	79.9	19.9	0.124	1.052
24 hours	2318506	76.7	23.1	0.125	1.063

Table 11. Base degradation of tirbanibulin

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

Table 12. Peroxide degradation of tirbanibulin

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

Table 13. Reduction degradation of tirbanibulin

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

DISCUSSION

Tirbanibulin is a microtubule inhibitor. Tirbanibulin is a non-ATP-competitive inhibitor that disrupts the proto-oncogenic Src tyrosine kinase signaling 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 ensure that it is suitable for a certain analysis.[6] The new approach was tuned to produce symmetrical peaks 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 limits. These findings demonstrate that the proposed strategy can produce data of acceptable quality. The suggested method's application to the analysis of formulations is a key feature. Hence, the market sample of tirbanibulin was collected and analyzed by employing the proposed method. The study confirmed that the created UPLC method was accurate and easy enough to be used daily. The suggested assay method's high content results indicate that this technique can be employed for quantitative regular quality control studies of pharmaceutical dosage forms.

Table 14. Thermal degradation of tirbanibulin

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

Table 15. Photolysis degradation of tirbanibulin

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

Table 16. Hydrolytic degradation of tirbanibulin

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

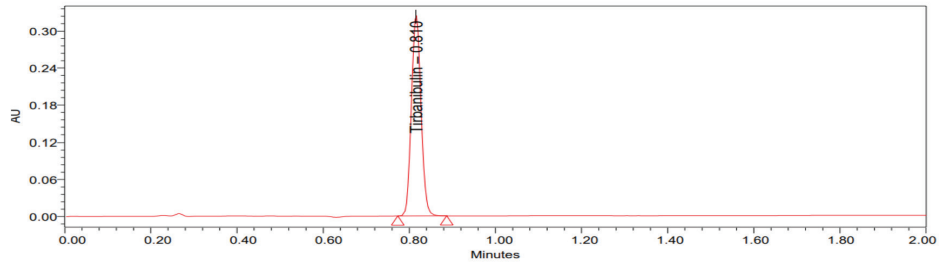

Figure 6. Acid degradation chromatogram of tirbanibulin: initial
AU: Absorbance units

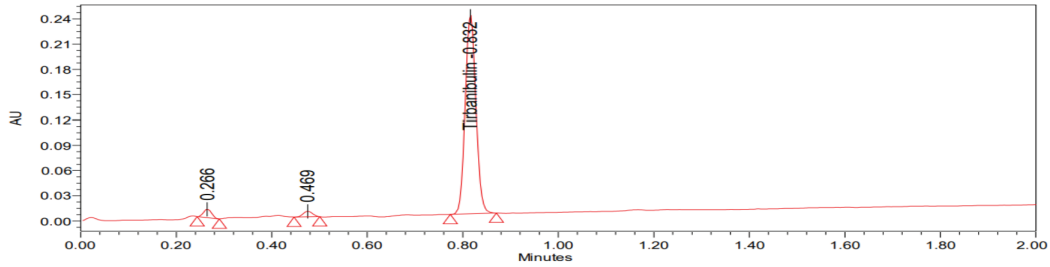

Figure 7. Acid degradation chromatogram of tirbanibulin at 24 hours
AU: Absorbance units

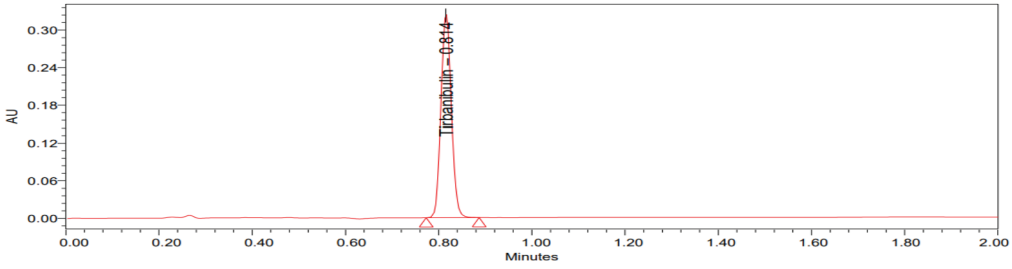

Figure 8. Base degradation chromatogram of tirbanibulin: initial
AU: Absorbance units

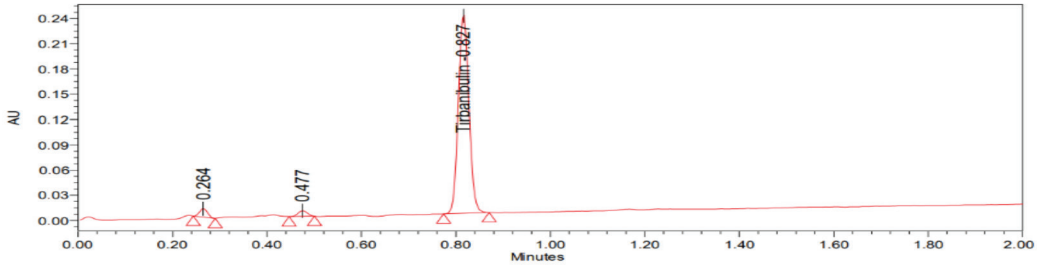

Figure 9. Base degradation chromatogram of tirbanibulin at 24 hours
AU: Absorbance units

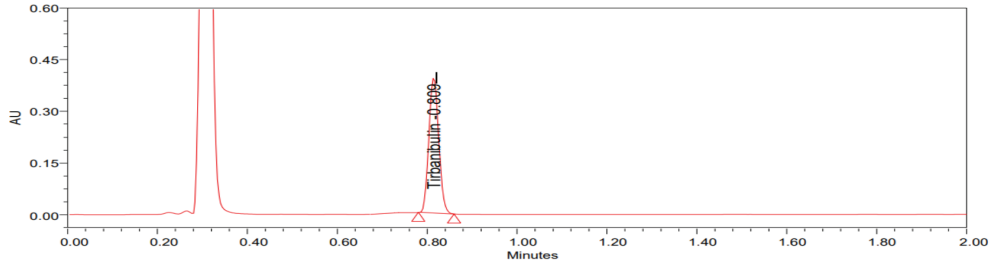

Figure 10. Peroxide degradation chromatogram of tirbanibul in: the initial
AU: Absorbance units

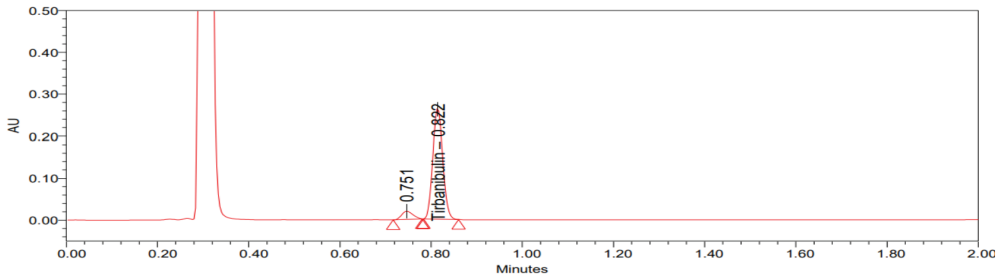

Figure 11. Peroxide degradation chromatogram of tirbanibul in: at 24 hours
AU: Absorbance units

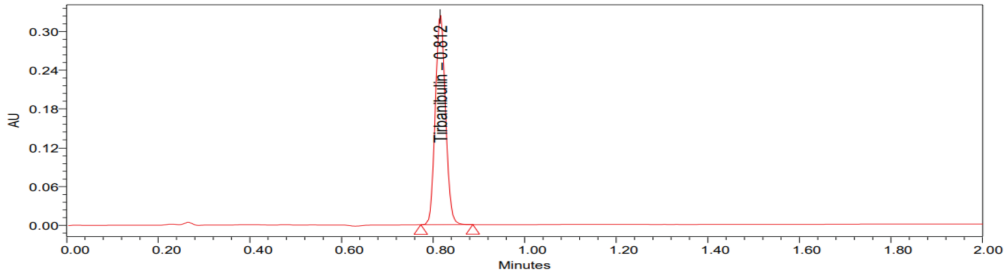

Figure 12. Reduction degradation chromatogram of tirbanibulin: initial
AU: Absorbance units

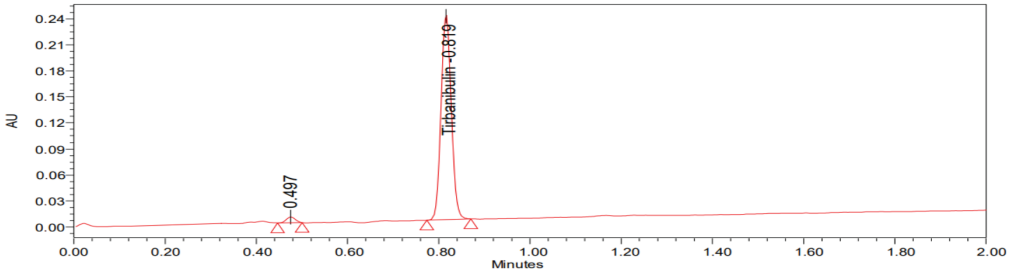

Figure 13. Reduction degradation chromatogram of tirbanibulin at 24 hours
AU: Absorbance units

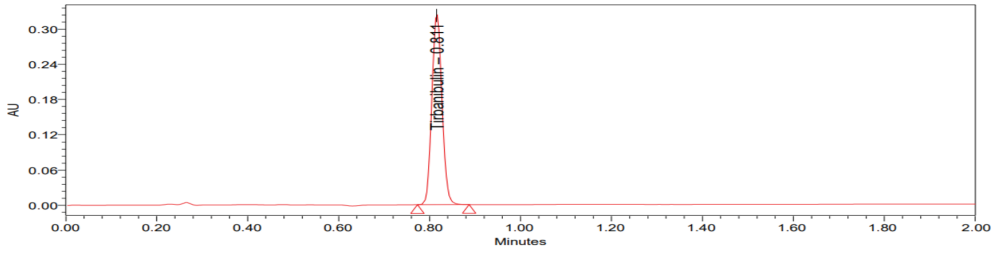

Figure 14. Thermal degradation chromatogram of tirbanibulin: initial
AU: Absorbance units

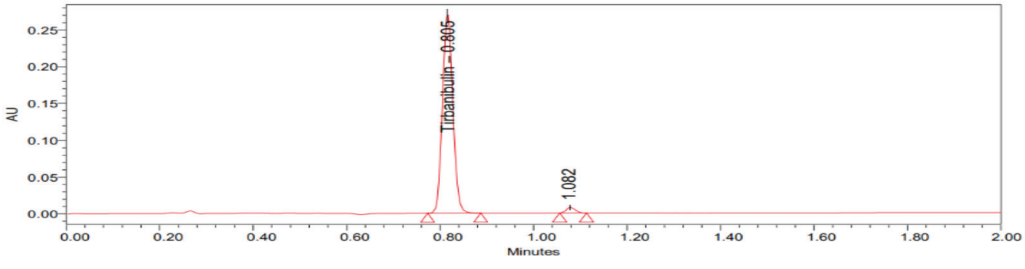

Figure 15. Thermal degradation chromatogram of tirbanibulin after 24 hours
AU: Absorbance units

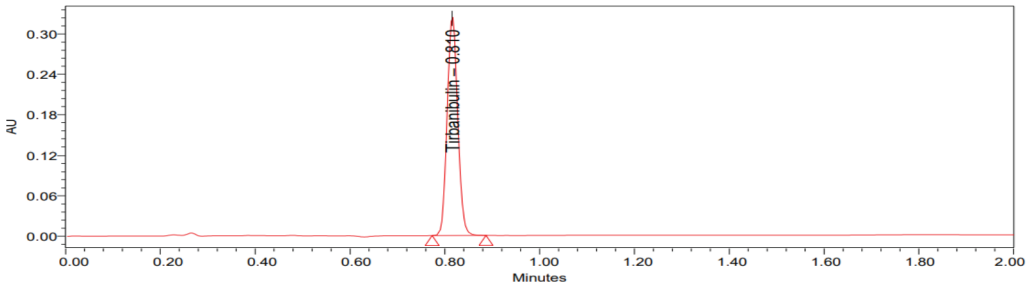

Figure 16. Hydrolysis degradation chromatogram of tirbanibul in: the initial
AU: Absorbance units

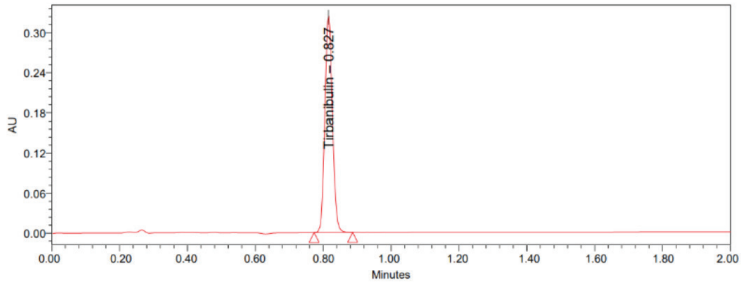

Figure 17. Hydrolysis degradation chromatogram of tirbanibulin after 24 hours
AU: Absorbance units

The linearity of the analytical method was it is capacity to produce test findings within a specified range that directly relates to the analyte concentration in the test sample.[6] The regression line of analysis expresses the relationship between the concentration and peak area of tirbanibulin. 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 the outcomes produced by that technique to the true value.[6] The percentage recovery and percent RSD results fell within the acceptable ranges of 98.0-102.0% and not more than 2.0%, respectively, demonstrating the suitability of the method for routine drug analysis. The degree of agreement between a set of measurements made using repeated samples of the same homogeneous material under specified conditions was considered the precision of the method, and it was typically stated as the RSD.[6] The results were well under the usually accepted 2% limit. As a result, the new method's precision has been confirmed. The LOQ was defined as the smallest amount of analyte in a sample that could be identified quantitatively with adequate accuracy. The lowest amount of analyte in a sample that could be detected but not necessarily quantitate was defined as the LOD.[6] The LOD and LOQ for tirbanibulin were 0.03 and 0.1 µg/mL, respectively. The system suitability parameters did not significantly change, 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 under peroxide degradation conditions. The tirbanibulin peaks were homogenous and showed results within acceptance limits in all forced degradation studies. This demonstrates that the proposed 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

To estimate tirbanibulin in dosage form, a simple, accurate, and specific approach was established. Tirbanibulin had a retention time of 0.811 min. The percent RSD of method precision and intermediate precision was found to be 0.47 and 0.33%, respectively. For tirbanbulin, the percent recovery was 100.1%. The LOD and LOQ values for tirbanibulin calculated from regression equations were 0.03 µg/mL and 0.1 µg/mL, respectively. The 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 economical, and it might be used in frequent quality control tests in industries.

Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: The present research work was based on sample analysis using the UPLC technique.

Authorship Contributions

Concept: P.K.G., R.S., Design: P.K.G., R.S., Data Collection or Processing: P.K.G., Analysis or Interpretation: P.K.G., Literature Search: P.K.G., Writing: P.K.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Fania L, Didona D, Di Pietro FR, Verkhovskaia S, Morese R, Paolino G, Donati M, Ricci F, Coco V, Ricci F, Candi E, Abeni D, Dellambra E. Cutaneous squamous cell carcinoma: from pathophysiology to novel therapeutic approaches. Biomedicines. 2021;9:171.
2. Cockerell CJ. Pathology and pathobiology of the actinic (solar) keratosis. Br J Dermatol. 2003;149(Suppl 66):34-36.
3. Shen Y, Ha W, Zeng W, Queen D, Liu L. Exome sequencing identifies novel mutation signatures of UV radiation and trichostatin a in primary human keratinocytes. Sci Rep. 2020;10:4943.
4. Niu L, Yang J, Yan W, Yu Y, Zheng Y, Ye H, Chen Q, Chen L. Reversible binding of the anticancer drug KXO1 (tirbanibulin) to the colchicine-binding site of β-tubulin explains KXO1's low clinical toxicity. J Biol Chem. 2019;294:18099-18108.
5. Dao DD, Sahni VN, Sahni DR, Balogh EA, Grada A, Feldman SR. 1% Tirbanibulin ointment for the treatment of actinic keratoses. Ann Pharmacother. 2022;56:494-500.
6. ICH Guideline. "Validation of analytical procedures: text and methodology," in Proceedings of International Conference on Harmonization, Topic Q2 (R1), Geneva, Switzerland: 2005.