Development of Stability Indicating, Validated Liquid Chromatographic Method for Estimation of Sarecycline in its Formulations

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ABSTRACT

A stability indicating HPLC method was developed and for the estimation of Sarecycline Hydrochloride in tablet dosage form using C_{18} column with a mobile phase composition of 0.1M Na_2PO_4 and Acetonitrile in the ratio of 50:50 v/v. The detection wave maxima and retention time were found to 242nm and 3.876 min respectively. The method validation was carried out according to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines and the parameters namely; precision, accuracy, specificity, stability, robustness, linearity, limit of quantitation (LOQ) and limit of detection (LOD) are evaluated. The present developed RP-HPLC method shows the purity angle of peaks is less than their threshold angle, signifying that it to be suitable for stability studies. Hence, the developed method can be used for the successful estimation of Sarecycline in the pharmaceutical dosage formulations.

Keywords: Sarecycline Hydrochloride, RP-HPLC, Formulation, Validation.

INTRODUCTION

The drug used in the present study is Sarecycline hydrochloride, chemically (4S,4a S,5aR,12aR)-4-(Dimethylamino)-1,10,11,12 a-tetrahydroxy-7-[[methoxy(methyl)amino]methyl]-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide and the structure was shown in Fig.1. It inhibits protein synthesis and other biosynthetic activities and shows anti-inflammatory action[1].

Sarecycline is a semi-synthetic derivative of tetracycline that was initially discovered by Paratek Pharmaceuticals from Boston, MA but then licensed to Warner Chilcott of Rockaway, NJ . After completing various phase-II and phase-III trials demonstrating its effectiveness in treating moderate to severe facial acne vulgaris the US Food and Drug Administration approved Barcelona based Almirall, S.A.'s Seysara (sarecylcine) as a new first in class narrow spectrum tetracycline derived oral antibiotic for the treatment

of inflammatory lesions of non-nodular moderate to severe acne vulgaris in patients nine years of age and older. Seysara (sarecycline) was originally part of Allergan's US Medical Dermatology portfolio, before Almirall acquired the portfolio in the second half of 2018 as a means of consolidating and reinforcing the dermatology-focused pharmaceutical company's presence in the United States"[2].

There are only limited reports regarding determination of Sarecycline in pharmaceutical dosage forms and biological fluids. Sarecycline is not official in any pharmacopoeia and there is no monograph containing methods to characterize or quantify Sarecycline. Such methods could offer official parameters to guarantee the validity of the assay. For the estimation of Sarecycline in its tablet dosage forms, reliable HPLC method is required to maintain the quality, potency and strength of the dosage forms to maintain safety, efficacy and bio-availability. There was no method found in the literature for the estimation of Sarecycline in tablets by HPLC, therefore Sarecycline requires effective and reliable method for quantitative estimation in tablet dosage form[3].
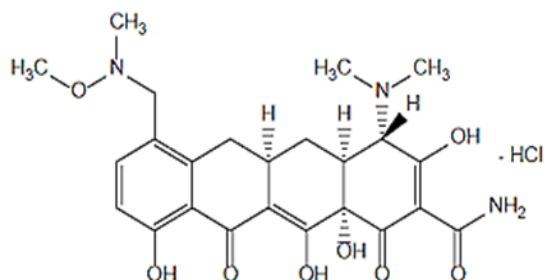

Fig. 1. Structure of Sarecycline Hydrochloride

MATERIALS AND METHODS

Equipment and Chemical reagents

WATERS Alliance High-performance liquid chromatography system (2695 model) with EMPOWER-2 software, equipped with Photodiode Array (PDA) detector was used throughout the analysis. All the chemicals and reagents used in this experiment were of analytical grade. High purified Millipore water from Milli Q water system was used. Acetonitrile used was obtained from Sd fine-Chem Limited, India. Disodium hydrogen phosphate, HCl, NaOH, H_2O_2, H_3PO_4 were purchased from Merck, India. Pharmaceutical grade API, Sarecycline

was gifted by Ven Life Sciences Pvt, Ltd. Tablet formulation SEYSARA (Sarecycline) tablets, 100 mg are capsule-shaped, yellow, film-coated tablets debossed with "S100" on one side purchased from local market of Hyderabad, Telangana State, India.

Chromatographic Settings

The mobile phase used for the development of method was Na_2HPO_4: ACN (50:50 v/v) with pH adjusted to pH 4.8 and the mobile phase was filtered through membrane filter and flow rate was maintained at 1mL/minute. The effluents were detected at 242 nm with PDA detector by injecting 10 µL of solution through chromatographic column.

Method development
Preparation of mobile phase

Mobile phase composition of disodium hydrogen phosphate and acetonitrile in the ratios of 50:50 (v/v) used.

Standard stock preparation

100 mg of the Sarecycline reference standard in 100 mL volumetric flask dissolve and make up the volume to 100 mL using mobile phase as diluent. From the above solution, 1 mL is taken to make up the volume 10 mL to get stock concentration of 100µg/mL.

Calibration plot

Calibration Curve was plotted by taking concentration on X-Axis and the area of peak on Y-Axis. Linearity was established in between 50-150 µg/mL by recording the chromatograms on HPLC using chromatographic conditions mentioned in the Table 1.

Working Sarecycline solutions for validation process

1 mL of stock Sarecycline solution is diluted to 10 mL with mobile phase, to obtain the working Sarecycline concentration of 100 µg/mL.

Estimation of drug in tablet formulation

Weigh and powder 20 Seysara tablets (Strength – 100 mg per tablet). Take weight equivalent to 100 mg Sarecycline in a 100 mL volumetric flask, dissolve and make up the volume to 100 mL to obtain 1000 µg/mL. Suitable aliquots were diluted to get the sample concentration in the linearity range that is 100 µg/mL and was analyzed using chromatographic conditions given in Table 1.

Validation
System Suitability

It is an integral part of various analytical procedures[4-6]. Standard solutions of Sarecycline 1000 µg/mL has been prepared and injected 5 replicates[7,8]. Suitability of system was proved from the properties of Sarecycline peak.

- Peak area (permitted value: RSD - ≤ 2%)
- Plate count (permitted value: 2000)
- Tailing factor (permitted value: ≤ 2%)

The values revealed that the system is best suited for evaluation of Sarecycline.

Linearity

Calibration solutions with concentrations of 50, 75, 100, 125 and 150 µg/mL were prepared and injected into HPLC system.

Accuracy

Recovery study is used to determine the accuracy of the sample. 50%, 100%, 150% concentration of Sarecycline was added to tablet solution that is pre-analyzed. Accuracy is assessed by using 9 determinations of minimum 3 concentrations[9,10].

Method Precision

It was obtained from the peak area obtained by the actual measurement of 6 replicates of fixed concentration[11,12]. Relative standard deviation was found to be less than 2%.

Robustness

Robustness for the drug is carried out bringing changes in the parameters like acetonitrile ratio in mobile phase (±5%), buffer pH(±0.1 units), flow rate (±0.1/min) and temperature (±2πc) and the peak properties of Sarecycline like tailing factor, plate count and peak area was examined.[13-15]

Selectivity

Selectivity assessment was carried out injecting 10 µl of standard Sarecycline solution (100 µg/mL), Sarecycline tablet solution (100 µg/mL), blank (diluent without drug) and placebo solution into system separately and chromatograms were recorded and checked for the presence of any other peaks arising from placebo or blank.

LOD & LOQ

Signal to noise ratio values for LOD and LOQ were found to be 3.08, 10.5 respectively. LOD, LOQ concentrations for Sarecycline were found to be 0.157 µg/mL, 0.523 µg/mL respectively.

Range

The range of analytical method is the interval between the upper and lower limits of the analyte determined by precision, accuracy and linearity[16,17]. Linearity was established from 50 to 150 µg/mL concentration by taking suitable aliquots from standard stock.

Specificity

It is the ability to identify the analyte in the presence degraded products[18].

Stability testing of Sarecycline

Stability testing is carried using tablet sample solution (1000 µg/mL) as per ICH criteria.[19,20]

Acid degradation

The drug is being subjected to acidic condition using hydrochloric acid with a theoretical concentration of 100 µg/mL and the degraded solution was analyzed by placing into the vials and injecting into the system and the degradation percent was obtained from the peak area values.

Alkali degradation studies

To initiate this, drug is treated with sodium hydroxide by using the concentration as 0.1 mg/mL, and have used for alkali stress testing.

Oxidative degradation

There are three major pathways for oxidative degradation to take place. In this stability indicating studies, peroxide solution has used.

Photolytic degradation

The drug in petri plate is subjected to sunlight & subjected to analysis.

Thermal degradation

Thermal degradation studies are carried out at elevated temperature in solid state by using hot air oven at elevated temperatures.

RESULTS AND DISCUSSION

Method development
Optimization of chromatographic conditions

Several mobile phase compositions and different stationary phases were investigated in the preliminary studies to get the best resolution. The analyte has the different retention behaviors and hence it is a challenging development, without interfering excipients. Based on the Design of Experiments (DOE), the final chromatographic conditions were optimized which gives a powerful suite for a statistical methodology and the obtained results were presented in Table 1 and Figure 2.

Table 1: Chromatographic conditions

Parameters	Conditions
Column	C_{18}(250mm×4.6mm×5μm)
Mobile Phase	Na_2HPO_4:ACN (50:50v/v) with pH adjusted to 4.8
Flow rate (ml/min)	1
Run time	6
Injection volume(μl)	10
Detection wavelength (nm)	242
R_t (min)	3.876 min
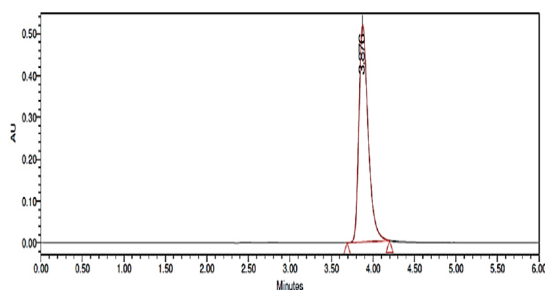
Fig. 2. Optimized standard chromatogram

Assay of marketed formulation

Standard and sample solutions are injected into HPLC separately to obtain chromatograms are recorded and the percentage of drug is calculated and the results were mentioned in the Table 2.

Table 2: Assay of formulation

S. no	Assay Percentage
1	99.25
2	99.43
3	99.26
4	99.38
5	99.30
6	99.29
Standard deviation	0.068
%RSD	0.07%

Method validation
Linearity

Different concentrations of calibration solutions were produced to study linearity[21,22]. R^2 for the Sarecycline Hydrochloride was 0.9998. The method found linear from 50 to 150 μg/mL Sarecycline. The data obtained for linearity study of Sarecycline is given in Table 3 and linearity plot was drawn as shown in the Figure 3.

Table 3: Sarecycline linearity

Calibration solution	Sarecycline (μg/ml)	Sarecycline peak area	Retention time
1	50	2066023	3.867
2	75	3096395	3.864
3	100	4123873	3.868
4	125	5150306	3.867
5	150	6186308	3.744
Equation		Peak area = 41217 x + 2424	
Regression coefficient		0.9998	
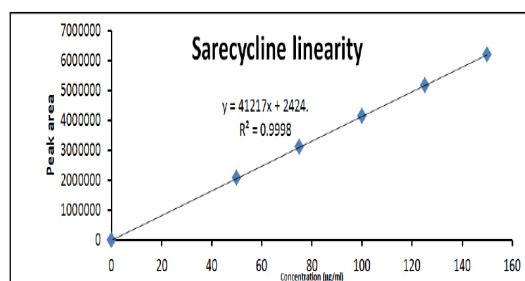
Fig. 3. Linearity of Sarecycline Hydrochloride

Accuracy

Recovery study was performed to determine the method accuracy at 49.5, 99.0 and 148.5 μg/mL (50, 100 and 150% levels) concentration of Sarecycline RS was added to tablet solution that is pre analyzed. For each concentration level, the percent recovery of Sarecycline added was determined .The values revealed that the method is accurate for evaluation of Sarecycline. Interference from tablet excipients was not found during evaluation of Sarecycline. Accuracy data and chromatograms were shown in the Table 4 and Figure 4 respectively.
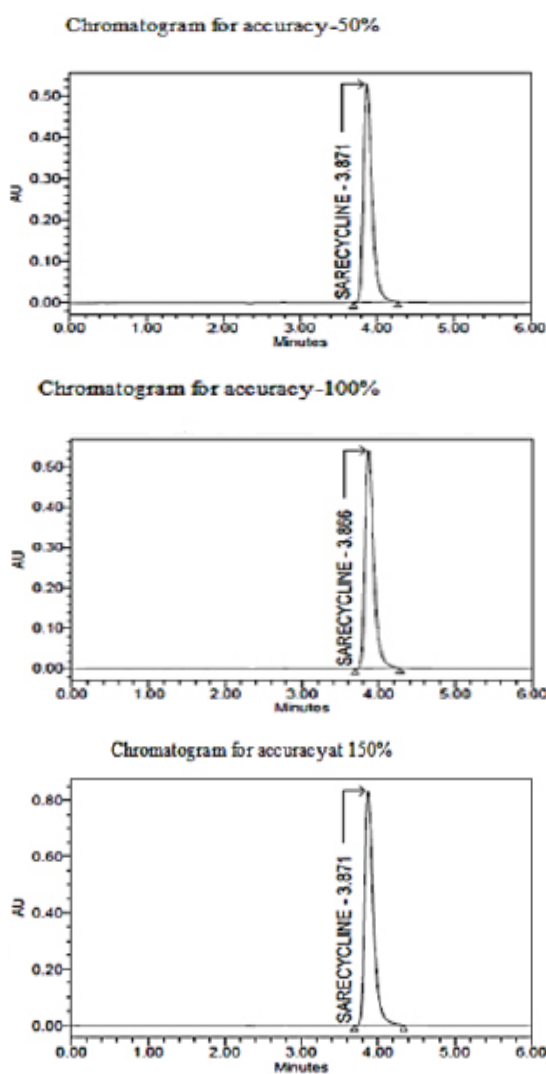
Precision

The precision data was determined using six replicates peak area of Sarecycline standard solution (100 mg/mL). Relative standard deviation of Sarecycline peak area is calculated and was less

than 2%. The values revealed that the method is precise for evaluation of Sarecycline. The precision

data is given in Table 5 and the chromatograms for precision is given in Figure 5.

**Table 4: Accuracy of Sarecycline Hydrochloride:**

Spiked level	Sarecycline Area	Sarecycline added(µg/ml)	Sarecycline found(µg/ml)	Sarecycline Recovered%	% Mean recovery
50% Level	2062429	49.500	49.66	100.31	100.27
	2060230	49.500	49.60	100.21	
	2061770	49.500	49.64	100.28	
100% Level	4126786	99.000	99.36	100.36	100.36
	4124933	99.000	99.31	100.32	
	4128309	99.000	99.39	100.40	
150% Level	6188322	148.500	148.99	100.33	100.30
	6183530	148.500	148.88	100.25	
	6187337	148.500	148.97	100.31	

Fig. 4. Chromatograms showing Accuracy at different concentration

Table 5: Precision data for Sarecycline

Sarecycline -100µg/ml	
Injection	Sarecycline peak area
1	4122502
2	4129745
3	4123040
4	4127866
5	4127814
6	4124015
Mean	4125830
Std deviation	3018.6979
%RSD	0.0732
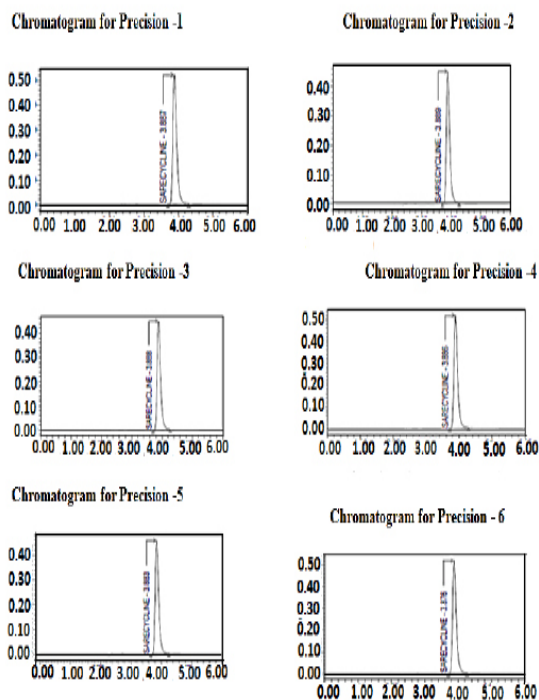
Fig. 5. Chromatograms for Precision

Robustness

Robustness was established by doing deliberate changes in flow rate and different pH values and the study data was given in the Table 6.

Table 6: Robustness data

| Alternation in condition	Tailing of peak	Area response	Plate count
Flow rate in column: 0.9 (ml/min)	1.32	3311701	4539
Flow rate in column:1.1 (ml/min)	1.33	5870815	5566
pH: 4.7 units	1.48	4536786	5973
pH: 4.9 units	1.31	4224933	5982
Acetonitrile portion in mobile phase: 45 (%)	1.41	4077561	6010
Acetonitrile portion in mobile phase: 55 (%)	1.34	3607336	5954
Temperature of column: 23 (°C)	1.30	3308037	4458
Temperature of column: 27 (°C)	1.34	5901970	5173

LOD and LOQ

LOD & LOQ were found to be 0.157, 0.523 µg/mL respectively & were found within limits and the study data and chromatograms were given in the Table 7 and Figure 6.

Table 7: LOD and LOQ for Sarecycline hydrochloride

S. No	Parameter	Retention time	Area	S/N
1	LOD	3.870	5320	3.08
2	LOQ	3.810	27961	10.35

Chromatogram for LOD
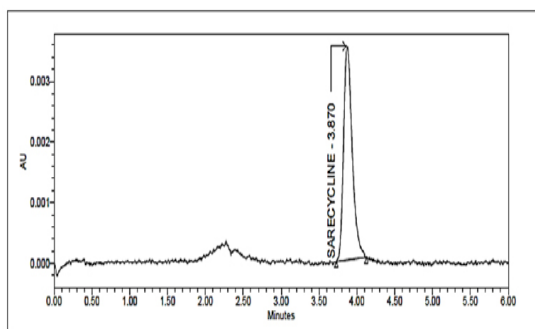
Chromatogram for LOQ
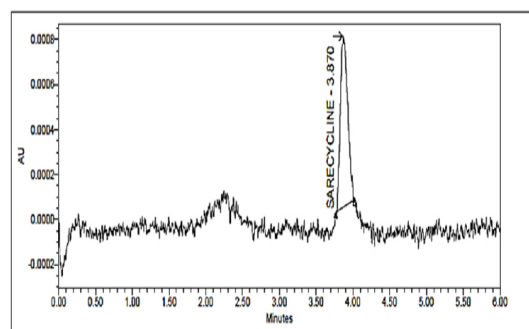
Fig. 6. Chromatograms showing the LOD and LOQ for Sarecycline Hydrochloride

Forced Degradation studies
Degradation by acid condition

10 mL of Sarecycline 1000 µg/mL tablet stock solution is taken in100 mL vol. flask and add 10 mL of 0.1N HCl, sonicate for 30 min at ambient temperature, neutralize with 0.1 NaOH and make up the volume to 100 mL volume with mobile phase[22]. This degraded sample solution was analyzed placed in vial and injected into system to obtain chromatogram. The degradation percent of Sarecycline was estimated from peak area values acquired has been added adequately and the chromatogram of acid degradation sample was shown in the Figure 7.
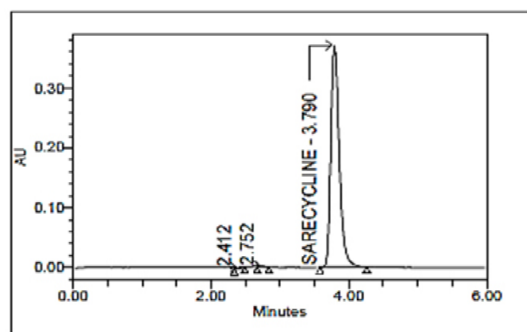
Fig. 7. Chromatogram for degradation by acid

Degradation by alkaline condition

10 mL of 1000 µg/mL Sarecycline tablet stock solution is taken in vol. flask (100 mL) and add 10 mL of 0.1 N NaOH and sonicated for 30 minute. Neutralize with 0.1N HCl and make up the volume with diluent and record chromatogram[23] and the chromatogram of alkaline degradation sample was shown in the Figure 8.
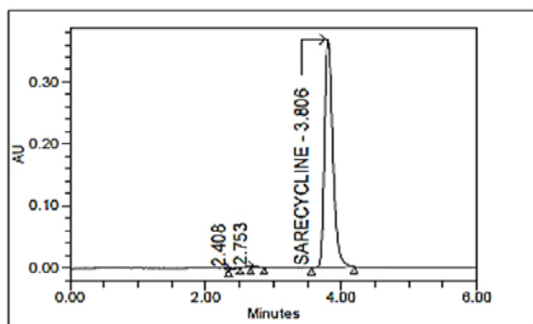
Fig. 8. Chromatogram for alkali degradation

Degradation by peroxide

10 mL of stock solution is mixed with 10 mL of 30% hydrogen peroxide (H_2O_2) and shaken continuously using sonicator for 30 min at ambient temperature and volume was made to 100 mL with 0.01 mg/mL as the theoretical concentration and was analyzed using given optimized chromatographic conditions and the degradant percent is acquired from peak area values and the chromatogram of peroxide degradation sample was shown in the Figure 9.
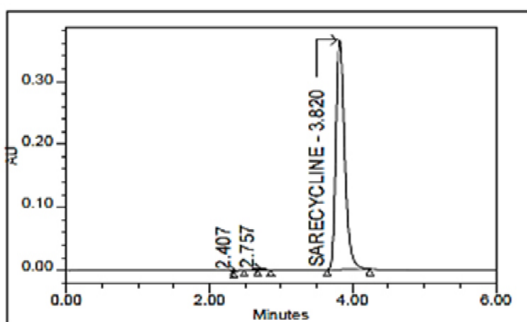

Fig. 9. Chromatogram for degradation by peroxide

Degradation by Heat

Powder form of tablet weighing 100 mg of Sarecycline API was taken into petri dish and positioned for 6 h in hot air oven at 105°C and sample was cooled down to bring down the temperature and transferred into standard flask (100 mL), dissolve & make up the volume to 100 mL. Similarly prepare 100μg/mL and analysis was carried and degradant percent is calculated from peak area and the chromatogram of thermal degradation sample was shown in the Figure 10.

Degradation by sunlight (Photolytic degradation)

Powder form of tablet weighing 100 mg is taken into petri plate placed for 6 h in sunlight the sample was brought to normal temperature and

transferred into standard volumetric flask (100 ml). After suitable dilution to get 100 μg/mL, the degradant percent is estimated and the chromatogram of photolytic degradation sample was shown in the Figure 11.
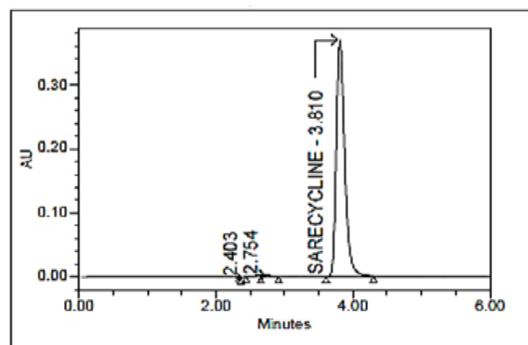

Fig. 10. Chromatogram for degradation by heat
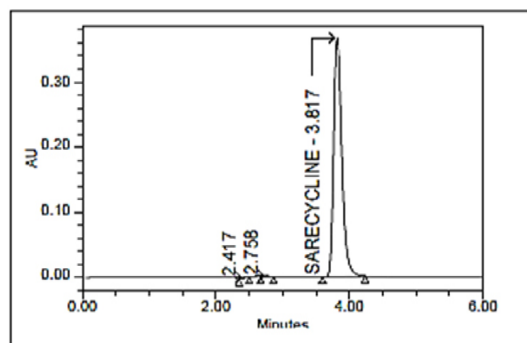

Fig. 11. Chromatogram for photolytic degradation

Degradation study of Sarecycline

The order of stability of Sarecycline is Peroxide, Sunlight, NaOH, HCl, Dry heat. Two degradants were observed and these degradant peaks were resolved good from the Sarecycline peak. These results indicated that the method is specific and stability indicating for evaluation of Sarecycline in vicinity of degradants. The forced degradation data was shown in the Tables 8, 9.

Table 8: Sarecycline stability data

Condition	Area	Sarecycline assay%	Sarecycline degraded%	Rt of degradation products
Sample without degradation	4120250	-	-	-
Alkali	3764973	90.65	9.35	2.408 min, 2.753 min
Acid	3697561	89.02	10.98	2.412 min, 2.751 min
Photo	3794840	91.37	8.63	2.417 min, 2.758 min
Dry heat	3664638	88.23	11.77	2.403 min, 2.754 min
Peroxide	3892725	93.72	6.28	2.407 min, 2.757 min

Table 9: Complete degradation data

S.No	Type of stress	Assay%	%Degradant	Rt	Purity angle	Purity threshold	Peak purity (pass/fail)
1	Acid	89.02	10.98	4.038	0.347	0.972	Pass
2	Base	90.65	9.35	4.006	0.455	0.775	Pass
3	Peroxide	93.72	6.28	4.015	0.331	0.871	Pass
4	Dry heat	88.23	11.77	4.020	0.346	0.872	Pass
5	Sunlight	91.7	8.63	4.028	0.234	0.971	Pass

A degradation data result shows that peak threshold is greater than peak angle indicating the method is sensitive and reliable.

not have interference of excipients. The proposed method could be useful for routine analysis in quality control labs.

CONCLUSION

An easy and speedy liquid chromatographic method to estimate the Sarecycline in tablets has been developed and validated for parameters such as sensitivity, precision, accuracy, robustness, specificity and linearity. The method was proven to be specific under stress conditions such as basic, acidic, oxidative, photo and thermal and do

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Conflict of Interests

The authors declare that they have no conflict of interest.

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