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Stability Indicating RP-HPLC Method for Quantification of Sarecycline and Its Impurities in Sarecycline Solid Dosage Form

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Abstract

The primary objective of the research work is to develop an effective, sensitive, economical and simple reverse phase HPLC method for quantification of Sarecycline and its impurities in Sarecycline parenteral dosage form. The separation was achieved by using a stationary phase water X-Bridge shield RP18 (150 x 4.6 mm, 3.5µ) and the mobile phase consists of ammonium acetate buffer and acetonitrile in the proportion of gradient elution. The flow rate was 1.0 mL/min. Sarecycline was detected by using UV detector at the wavelength of 240 nm. The column temperature was maintained at 40°C and sample cooler temperature was maintained at 5°C, injection volume 10µL, run time was 45 minutes. The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, solution stability.

Keywords: Sarecycline, Liquid chromatography, Related substances, Assay, Forced degradation and Validation.

Introduction

The chemical name of Sarecycline is (4S,4aS,5aR,12aR)-4-(Dimethylamino)-1,10,11,12a-tetrahydroxy-7-((methoxy(methyl)amino)methyl)-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide corresponding to the molecular formula $C_{24}H_{29}N_3O_8$. It has a relative molecular mass of 487.509 g/mol. The chemical structure of Sarecycline was shown in Figure 1.

Sarecycline, sold under the brand name Seysara, is a narrow-spectrum tetracycline-derived antibiotic medication. It is specifically designed for the treatment of acne, and was approved by the FDA in October 2018 for the treatment of inflammatory lesions of non-nodular moderate to severe acne vulgaris in patients 9 years of age and older. Two randomized and well-controlled clinical trials reported efficacy data on both facial and truncal acne (back and chest). Efficacy was assessed in a total of 2002 subjects 9 years of age and older. Unlike other tetracycline-class antibiotics, Sarecycline has a long C7 moiety that extends into and directly interacts with the bacterial messenger RNA (mRNA). The spectrum of activity is limited to clinically relevant Gram-positive bacteria, mainly Cutibacterium acnes, with little or no activity against Gram-negative bacterial microflora commonly found in the human gastrointestinal tract (1-6).

Mechanism of action

It has been demonstrated that tetracyclines like Sarecycline elicit their antimicrobial action by targeting and inhibiting protein synthesis in microbial agents like Cutibacterium acnes

present in acne lesions (1,2,3). In particular, it is believed that sarecycline's mechanism of action revolves around the inhibition of various macromolecular biosynthesis activities like the macromolecular biosynthesis of microbial DNA, RNA, proteins, lipids, and cell wall. Specifically, it has been observed that while Sarecycline demonstrates appreciable inhibition of microbial macromolecular DNA and protein synthesis, the compound has little to no effect on lipid biosynthesis, cell wall synthesis, and RNA synthesis. In addition, because Cutibacterium acnes also generates proteins and enzymes that are capable of causing inflammation, it is also believed that tetracyclines like sarecyclines can also affect an anti-inflammatory effect via the inhibition of such microbial protein synthesis.

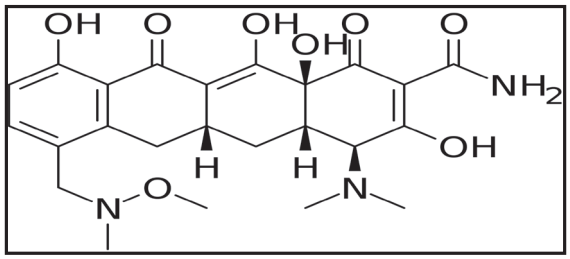

Figure 1: Chemical structure of Sarecycline

The literature survey reveals that only a few methods were reported to date for the estimation of Sarecycline in HPLC and biological fluid were carried out by LC-MS/MS (11) method. Hence we tried to develop stability indicating the HPLC method for quantification of Sarecycline and its impurities in Sarecycline solid dosage form according to ICH guidelines (12-13).

Materials and Methods

Chemicals and reagents

Ammonium acetate (AR grade), Acetonitrile (HPLC grade), Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and water, reagents and chemicals were procured from merck chemicals. Mumbai, India.

Instrumentation

Waters HPLC model: e2695 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model) and Analytical Balance (Metller Toledo Model) were used in the present study.

Preparation of mobile phase-A

Accurately weighed and transferred 0.7754 g of ammonium acetate into a 1000 mL of milli-Q water and mixed well. Filtered the solution with 0.45 µm membrane filter and sonicate to degas.

Preparation of mobile phase-B

Prepared a mixture of 900 mL of Acetonitrile and 100 mL of water in the ratio of 90:10 (%v/v). Filtered the solution with 0.45 µm membrane filter and sonicate to degas.

Preparation of diluents

Prepared a mixture of 500 mL of water and 500 mL of acetonitrile in the ratio of 50:50 (%volume/volume). Filtered the solution with 0.45 µm membrane filter and sonicate to degas.

Preparation of standard solution

Weighed accurately 10.25 mg of Sarecycline working standard in to a10 mL volumetric flask, to it added 5mL of diluent sonicate to dissolve and diluted to volume with diluent and mixed well. Further transferred 2.0mL of this solution into a 100 mL volumetric flask, diluted to volume with diluent and mixed well. Further transferred 5.0 mL of this solution into a 50mL volumetric flask, diluted to volume with diluent and mixed well. (The concentration of the solution contains 0.002 mg/ mL of Sarecycline).

Preparation of placebo solution

Weighed accurately 3.87 mg of Sarecycline placebo (equivalent to 50 mg of Sarecycline) into a 50 mL volumetric flask, added 25mL of diluent and sonicated for 30minutes with intermediate shaking, maintaining the temperature at 25°C to dissolved and then diluted to the volume with diluent, mixed well. Filtered the

solution through 0.45µm PVDF syringe filter PVDF syringe filter.

Preparation of test solution

Weighed 10 tablets, take average weight and crushed into fine powder. Weighed accurately 53.87 mg (equivalent to 50 mg of Sarecycline) of Sarecycline sample powder, transferred into 50 mL volumetric flask, added 25mL of diluent, sonicated for 30 minutes with intermediate shaking, maintaining the temperature at 25°C to dissolved and then diluted to the volume with diluent, mixed well. Filtered the solution through 0.45 µm PVDF syringe filter.

Method development

UV-spectroscopic analysis of Sarecycline drug substance showed maximum UV absorbance (λmax) at 240 nm respectively.

To develop a suitable and robust HPLC method for the quantification of Sarecycline and its impurities in Sarecycline solid dosage form, different mobile phases were employed to achieve a efficiently quantification of Sarecycline and separation of impurities from blank, placebo and Sarecycline analyte peak.

The method development was started with waters x-bridge shield RP-18 (150 x 4.6 mm, 3.5µ) with the following different mobile phase compositions like mobile phase-A 0.1% perchloric acid buffer and mobile phase-B acetonitrile in gradient mode. There was no proper resolution of impurities and analyte peak and efficiency of the peak is also not achieved and peak interferences are present.

For the next trial the mobile phase consisted of pH 2.8 phosphate buffer and acetonitrile in gradient mode respectively, flow rate 1.0 mL/min, column temperature 25°C and sampler cooler maintained 5°C. UV detection was performed at 240nm. There was no proper resolution of impurities and analyte peak.

For the next attempt the mobile phase consisted of ammonium acetate buffer and acetonitrile and water in the ratio of (90:10 v/v) in gradient mode respectively, flow rate 1.0 mL/min, column temperature 40°C and sampler cooler maintained 5°C. UV detection was performed at 240nm. The resolution of both drug and impurities was achieved. These chromatographic conditions were selected for validation studies.

Optimised chromatographic conditions

Chromatographic analysis was performed on waters 2695 HPLC system. The chromatograms are recorded and analysed Empower[3] software. The separation was performed on waters x-bridge shield RP-18 (150 x 4.6 mm, 3.5µ) mobile phase consisting of mobile phase-A is ammonium acetate buffer and mobile phase-B is acetonitrile and water in gradient mode. The HPLC gradient program was time (min)/B% v/v: 0/10, 10/45, 20/45, 25/90, 35/90, 40/10 and 45/10. The flow rate was 1.0 mL/min, the column oven temperature was 40°C and the sampler cooler temperature was 5°C, the injection volume was 10µL, and detection was performed at 240 nm using a photodiode array detector (PDA).

Results and Discussion

(Related substances)

The developed RP-HPLC method was extensively validated for quantification of Sarecycline and its impurities in Sarecycline parenteral dosage form using the following parameters.

Specificity *(Blank and placebo interference)*

Specificity was demonstrated by injected blank solution, placebo solution, standard solution, sample solution and analyzed as per the optimised method. It was observed that impurities are not co eluting with each other and main analyte peak. Chromatogram of blank solution Figure 2. showed no peak at the retention time of Sarecycline analyte peak. Similarly chromatogram of the placebo solution Figure 3. showed no peaks at the retention time of Sarecycline analyte peak.

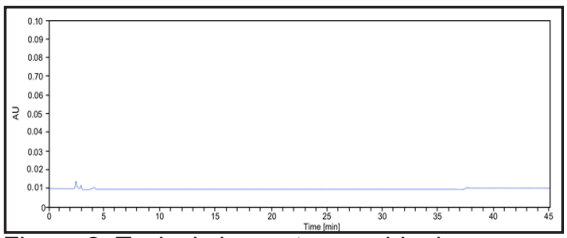

Figure 2: Typical chromatogram blank

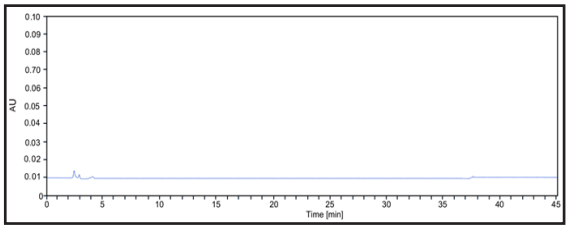

Figure 3: Typical chromatogram placebo

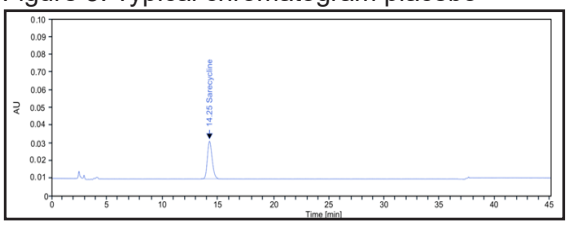

Figure 4: Typical chromatogram standard

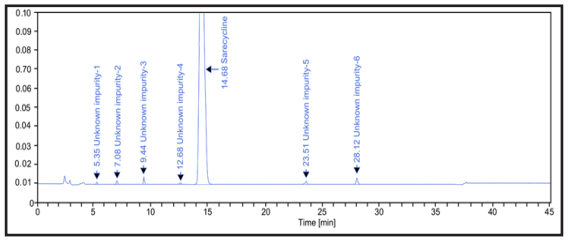

Figure 5: Typical chromatogram sample

Table 1: Specificity results

S. No.	Name	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	14.25	No	No
4	Sample solution	14.68	No	No

Table 2: System suitability results

S. No.	Name	Retention Time (min)	Theoretical plates	Tailing factor
1	Standard solution	14.25	11546	1.0

Force degradation studies

A study was conducted to demonstrate the effective separation of degradants/impurities from Sarecycline analyte peak. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the HPLC system with a PDA detector. The degradation study results were presented in Table 3 and Table 4.

Table 3: Forced degradation results

Stress condition	**Impurity at RRT about 1.60 (%)**	**Impurity at RRT about 1.91 (%)**	**Any single impurity (%)**	**Total impurities (%)**
As such	0.02	0.04	0.05	0.29
Acid	0.02	0.04	0.05	0.57
Alkali	0.02	0.04	0.06	0.45
Oxidative	4.11	0.04	0.05	4.81
Photolytic	0.03	3.97	0.06	5.25
Humidity	0.02	0.04	0.05	0.31
Thermal	0.02	0.03	0.06	0.35

Based on the above forced degradation results major degradation impurities are observed at RRT about 1.60 and 1.91 in the oxidation and photolytic stress conditions.

Table 4: Mass balance results

Stress condition	**Degradation condition**	**% Assay**	**% Degradation**
As such	Control sample	100.8	0.29
Acid	5.0 N HCl/ 60°C/24 hrs	99.7	0.57
Alkali	5.0 N NaOH/ 60°C/24 hrs	100.1	0.45
Oxidative	30% H_2O_2/ BT/24 hrs	95.5	4.81
Photolytic	(200 watt hours /m2 & 1.2 million Lux hours)	94.7	5.25
Humidity	90%RH exposed for 7 days	100.3	0.31
Thermal	105°C/7 days	100.5	0.35

Significant degradation was observed in the oxidation and photolytic stress conditions. Hence it can be concluded that Sarecycline is sensitive to oxidation and photolytic stress conditions.

System precision

The standard solution was prepared as per the optimised method, injected into the HPLC system six times, and evaluated the % RSD for the area responses. The data were shown in Table 5.

Table 5: System precision results

S.No.	No. of injections	Peak area
1	Inj-1	35560
2	Inj-2	36617
3	Inj-3	35730
4	Inj-4	36419
5	Inj-5	36195
6	Inj-6	36474
Avg.		36166
Std.Dev.		429.152485
%RSD		1.19

The %RSD of peak area for Sarecycline standard was found to be 1.19% which is below 5.0% indicates that the system gives precise result.

Method precision

Method precision was demonstrated by preparing six samples of Sarecycline 150 mg tablets as per method and injected in to the chromatographic system. The precision of the method was evaluated by calculating the impurities found and % relative standard deviation for impurities found for each set of samples. The results of the precision study are tabulated below Table 6.

Table 6: Method precision results

Preparations	Individual maximum unknown impurity (%)	Total impurities (%)
Prep-1	0.051	0.29
Prep-2	0.055	0.31
Prep-3	0.056	0.28
Prep-4	0.050	0.29
Prep-5	0.059	0.33
Prep-6	0.057	0.28
Average	0.05	0.30
STDEV	0.0035	0.0197
% RSD	6.41	6.63

The results were well within the limits. From the above results, it is concluded that method is precise.

Results and Discussion (Assay)

Preparation of standard solution

Weighed accurately 50.35 mg of Sarecycline working standard in to a 50 mL volumetric flask, to it added 25 mL of diluent sonicate to dissolved and diluted to volume with diluent and mixed well. Further transferred 5.0 mL of this solution into a 50 mL volumetric flask, diluted to volume with diluent and mixed well. (The concentration of the solution contains 0.1 mg/ mL of Sarecycline).

Preparation of test solution

Weighed 10 tablets, take average weight and crushed into fine powder. Weighed accurately 53.87 mg (equivalent to 50 mg of Sarecycline) of Sarecycline sample powder, transferred into 50 mL volumetric flask, added 25mL of diluent, sonicated for 30 minutes with intermediate shaking, maintaining the temperature at 25°C to dissolved and then diluted to the volume with diluent, mixed well. Filtered the solution through 0.45 µm PVDF syringe filter. Further transferred 5.0 mL of filtered solution

into a 50 mL volumetric flask, diluted to volume with diluent and mixed well. (The concentration of the solution contains 0.1 mg/ mL of Sarecycline).

Specificity *(Blank and placebo interference)*

Specificity was demonstrated by injected blank solution, placebo solution, standard solution, sample solution and analyzed as per the optimised method. Chromatogram of blank solution Figure 6. Showed no peak at the retention time of Sarecycline analyte peak. Similarly, chromatogram of the placebo solution Figure 7. Showed no peaks at the retention time of Sarecycline analyte peak.

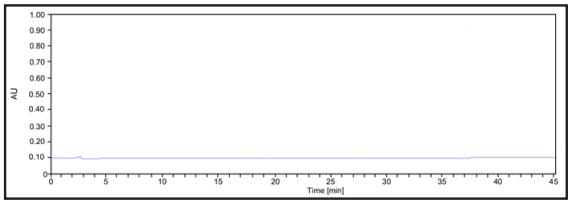

Figure 6: Typical chromatogram blank

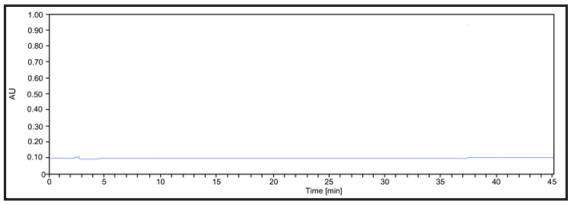

Figure 7: Typical chromatogram placebo

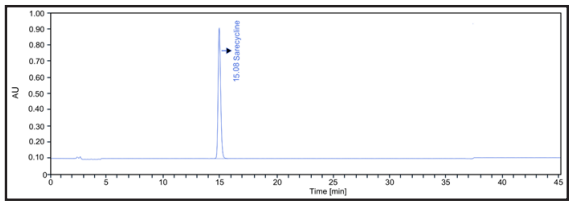

Figure 8: Typical chromatogram standard

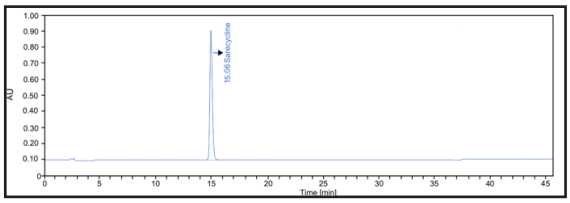

Figure 9: Typical chromatogram sample

System precision

The standard solution was arranged as per the test technique, infused keen on the HPLC system six times, and calculated the % RSD for the vicinity responses. The statistics were revealed in Table 7.

Table 7: System precision results

S.No.	No.of injections	Peak area
1	Inj-1	362712
2	Inj-2	373493
3	Inj-3	360873
4	Inj-4	371110
5	Inj-5	368103
6	Inj-6	370576
Average		367811
STDEV		5000.716636
% RSD		1.36

The relative standard deviation of six replicates standard solution consequences were establish to be within the specification limit i.e.1.36%.

Method precision

The method precision of the test method was estimated by doing an assay for six samples of Sarecycline 150 mg tablets as per the optimised technique. The % assay for Sarecycline for each of the test preparation was calculated. The middling content of the six arrangements and % RSD for the six observations were determined. The statistics were revealed in Table 8.

Table 8: Method precision results

S.No	No. of Preparations	% Assay
1	Preparation 1	100.8
2	Preparation 2	100.3
3	Preparation 3	100.7
4	Preparation 4	99.9
5	Preparation 5	100.9
6	Preparation 6	100.4
Average		100.5
SD		0.3742
%RSD		0.37

Linearity

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of the analyte. The linearity of response for Sarecycline was determined in the range of 50% to 150 % (50.35-151.05 µg/mL for Sarecycline). The statistics were revealed in Table 9.

Table 9: Linearity studies for Sarecycline

S. No	Linearity Level	Concentration (ppm)	Area response
1	50	50.35	183178
2	80	75.525	273515
3	100	100.7	364538
4	120	125.875	455776
5	150	151.05	547005
Correlation coefficient (r^2)			1.000
Slope			3614.3595
Intercept			836.4000
% Y-intercept			0.23

The linearity results for Sarecycline in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.9999.

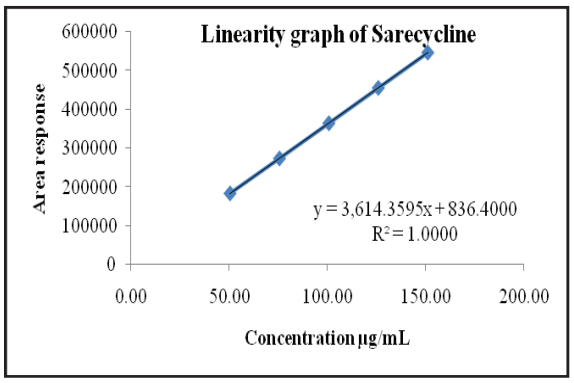

Figure 10: Linearity graph of Sarecycline

Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples at 50%, 100 % and 150 %of the target concentration level. The recovery samples were prepared in triplicate for each concentration level. The above samples were injected and the percentage recovery of each sample was calculated for the amount added. Evaluated the precision of the recovery at each level by computing the % Relative Standard Deviation of triplicate recovery samples results. The data obtained which given in **Table 10.** and the method was found to be accurate.

Table 10: Recovery studies for Sarecycline

Level	Added (µg)	Found (µg)	% Recovery	Mean % Recovery	%RSD
Accuracy at 50% Pre-1	50.2541	50.9945	101.47	101.0	0.54
Accuracy at 50% Pre-2	50.1846	50.7346	101.10		
Accuracy at 50% Pre-3	50.4327	50.6352	100.40		
Accuracy at 100% Pre-1	100.5246	100.3124	99.79	100.0	0.37
Accuracy at 100% Pre-2	100.3165	100.7415	100.42		
Accuracy at 100% Pre-3	100.6523	100.4178	99.77		
Accuracy at 150% Pre-1	150.6537	150.7452	100.06	100.0	0.10
Accuracy at 150% Pre-2	150.3418	150.4546	100.08		
Accuracy at 150% Pre-3	150.1545	149.9875	99.89		

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Solution stability of analytical solutions

Solution stability of standard, sample solutions were established at various conditions such as bench top on room temperature and at refrigerator 2-8°C. The stability of standard, sample solutions was determined by comparison of initially prepared standard, sample solutions with freshly prepared standard solution. The data obtained which given in Table 11 and 16.

Table 11: Solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.03	1.00
48hrs	1.05	1.02

Table 12: Solution stability of RS sample at room temperature

Component	Initial	After 24Hrs	% Difference	After 48Hrs	% Difference
Individual maximum unknown impurity (%)	0.051	0.055	0.04	0.059	0.08
Total impurities	0.29	0.31	0.02	0.35	0.06

Table 13: Solution stability of RS sample in refrigerator

Component	Initial	After 24Hrs	% Difference	After 48Hrs	% Difference
Individual maximum unknown impurity (%)	0.051	0.052	0.01	0.055	0.04
Total impurities	0.29	0.29	0.00	0.31	0.02

Table 14: Solution stability of Assay standard

Time Interval	**Similarity factor**	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.00	1.00
48hrs	1.01	1.00

Table 15: Solution stability of Assay sample at room temperature

Time Interval	**%Assay**	**%Assay difference**
Initial	100.8	NA
24hrs	100.5	0.3
48hrs	100.2	0.6

Table 16: Solution stability of Assay sample in refrigerator

Time Interval	**%Assay**	**%Assay difference**
Initial	100.8	NA
24hrs	100.5	0.3
48hrs	100.4	0.4

Standard and sample solutions are stable upto 48 hours when stored at room temperature and 2-8°C in refrigerator.

Conclusion

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity and

solution stability. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Sarecycline in bulk and pharmaceutical dosage forms.

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Conflict of Interests

The authors claim that there is no conflict of interest.

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