Amoxicillin Trihydrate Stability in Correlation with Coral Ointment Batch#18006-B and Simulated Seawater

Prepared By:

Mike Favero, Katie Balut, & Mark Levine

CoreRx Pharmaceuticals
14205 Myerlake Circle
Clearwater, FL 33760

June 24, 2019

Completed in Fulfillment of PO B52629 for

Florida Department of Environmental Protection
Coral Reef Conservation Program
1277 N.E. 79th Street Causeway
Miami, FL 33138

This report should be cited as follows:
Favero M, Balut K. 2019. Amoxicillin Trihydrate Stability in Correlation with Coral Ointment Batch#18006-B and Simulated Seawater. Florida DEP. Miami, FL. Pp. 1-9.

This report was prepared for the Florida Department of Environmental Protection (DEP), Office of Resilience and Coastal Protection by CoreRx Pharmaceuticals. Funding was provided by the Florida Department of Environmental Protection. The views, statements, findings, conclusions and recommendations expressed herein are those of the authors and do not necessarily reflect the views of the State of Florida or any of its sub-agencies.

Abstract:

The degradation of Amoxicillin Trihydrate suspended into a novel coral ointment formulation for underwater application was investigated in this study to determine the stability of the active ingredient over a period of 1 month. Degradation of Amoxicillin Trihydrate proved to be very minimal a rate of less than 2% per day. Based on this study, it can be determined that the excipients used in the ointment formulation do not appear to have a negative effect on the stability of the active ingredient once deployed in seawater. It should be noted that due to extraction issues, the degradation of API (active pharmaceutical ingredient-Amoxicillin Trihydrate) in dry ointment (ointment without the presence of seawater) yielded inconclusive results. The collected data set suggests a degradation rate of approximately 2% per day as well, however as complete recovery was never achieved, the definitive degradation rate cannot be conclusively proven.

Introduction:

The Florida Reef Tract has been subjected to a deadly coral disease known as Stony Coral Tissue Loss Disease (SCTLD) since late 2014. Ongoing research suggests treatment of disease lesions with Amoxicillin may be an effective means of slowing or stopping disease progression across individual coral colonies, although repeat treatments are often necessary as new lesions emerge. Initial supporting studies for this hypothesis were done so in controlled conditions, which allowed a tank bound body of water to be spiked with Amoxicillin. Corals were then exposed to these conditions for period of time, allowing them to be effectively medicated in the process.

Unfortunately, medicating corals by dosing the water column in their natural habitat is an impossibility. To test if Amoxicillin may be an effective treatment for wild corals, a new drug delivery system was needed. Researchers associated will CoreRx Pharmaceuticals were tasked with the challenge. An unconventional prototype was developed that proved to be capable of both adhering to skeletal coral and providing a slow diffusion rate paired with a mucosal adhesive which allowed for targeted delivery to the living coral tissue.

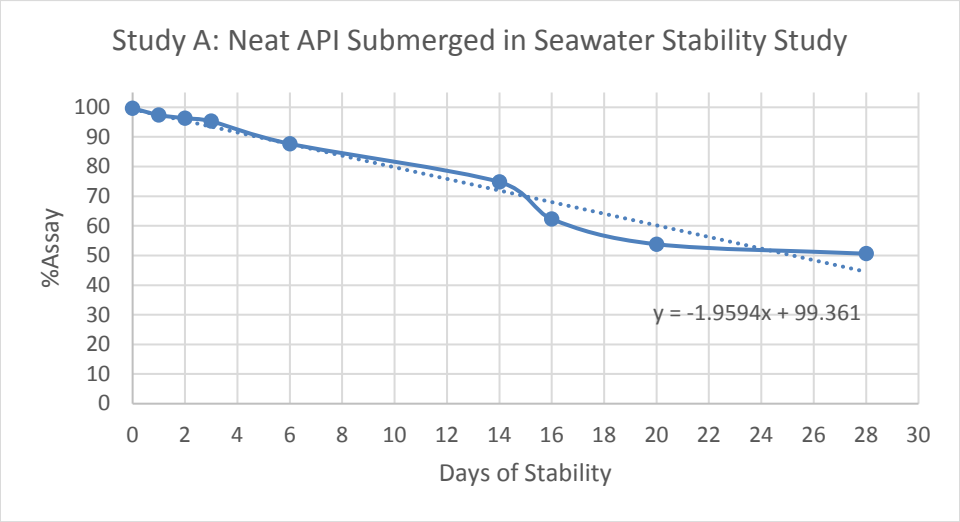
To date, the drugs stability both within the delivery system as well as in the water column were unknown. This study was initiated to better understand these attributes.

Results:

Amoxicillin in both simulated seawater and the delivery system (Coral Ointment Batch#18006-B) were tested and analyzed by HPLC (High Performance Liquid Chromatography). Instrument methods utilized modified USP (United States Pharmacopia) methodization for Amoxicillin Trihydrate. All samples were monitored for approximately 1 month in an effort to better understand the drugs stability in multiple environments. The study design saw a multitude of samples created over an extended time period. This allowed researchers to analyze Amoxicillin Trihydrate's stability in varied conditions.

Study A- Neat Amoxicillin Trihydrate in Seawater

Neat Amoxicillin stability in seawater exceeded expectations. The study saw individual samples created using either 25ml or 50ml volumetric flasks containing Amoxicillin Trihydrate in seawater at concentrations of approximately 1.25mg/mL. Results were quantified using HPLC assay values, where each sample was compared to a known standard concentration. Amoxicillin weights for each sample were saved and known which allowed for an accurate quantification of the concentration of Amoxicillin within each sample at their respective time point of creation. HPLC peak area count was then utilized to accurately quantitate the amount of Amoxicillin still present in each sample at the time of testing. Results suggest Amoxicillin degrades slowly, following a profile of approximately 2% degradation per day over a time period of 28 days.



Graph 1: Study A Stability Results-This graph represents ~2% degradation of the API/day in simulated seawater.

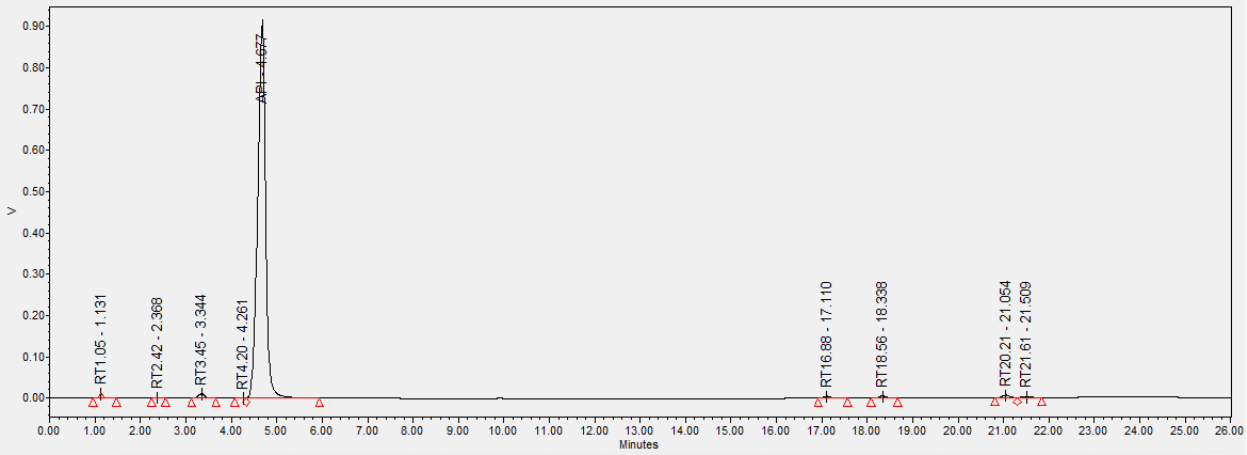


Image 1: Study A Initial Chromatogram-This graph represents the amount of API visible at 260nm in simulated seawater at the initial point of the stability study, with limited impurities.

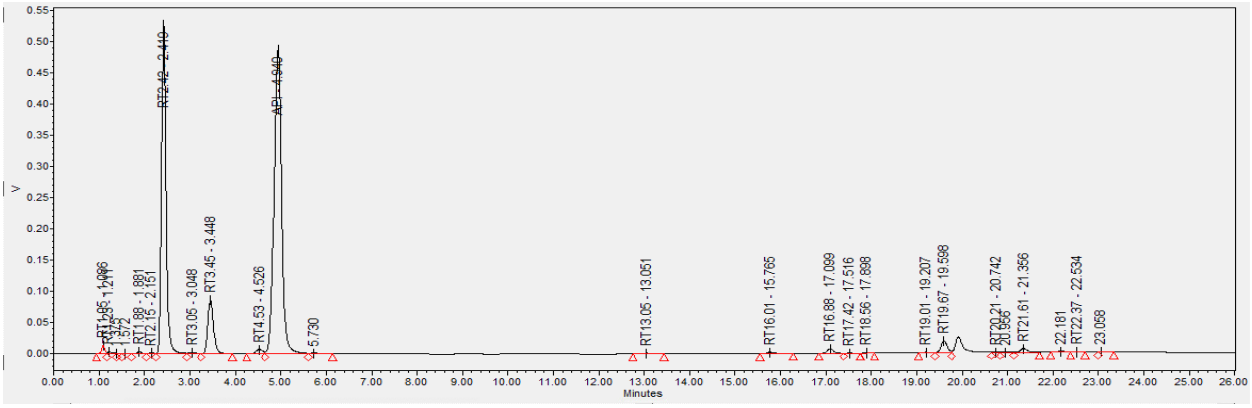
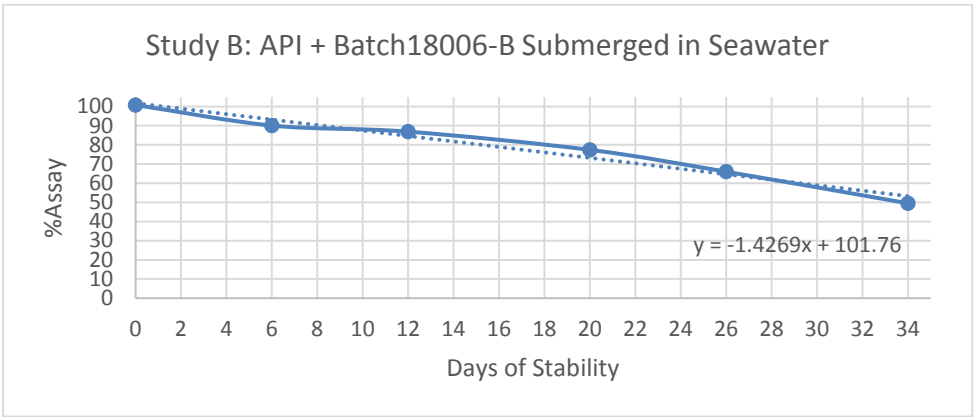


Image 2: Study A, 1 Month Stability Chromatogram- This graph represents the amount of API and all other visible impurities at 260nm that are produced during degradation of the API in simulated seawater at the end of 1 month.

Study B- Amoxicillin Trihydrate & Base 18006-B in Seawater

The study also monitored Amoxicillin's stability in relation to the drug delivery system in the presence of seawater. This was done to better understand how the delivery system impacts Amoxicillin's seawater bound stability. Results may be compared with those gathered from the neat Amoxicillin in seawater (Study A) to deduce the delivery system's effects. To do so, scintillation vials were filled with 15ml of simulated seawater. Amoxicillin Trihydate was dissolved into the seawater at a concentration of 2.5mg/mL. This was followed by the addition of 950mg of placebo drug delivery system known as Batch#137-18006-B (otherwise known as Base2B). Both the Amoxicillin and the placebo delivery system were added at elevated concentrations to better simulate localized conditions of a successfully adhered field application. The Amoxicillin and delivery system were added separately to improve chances of a full Amoxicillin extraction from each stability time point. As the results were again analyzed by assay, the utilized sample preparations decrease the possibility of any inaccuracies. Results of the study reveal Amoxicillin to degrade at approximately 1.5% per day over the course of the time period monitored. This correlates to a degradation rate slowing of approximately 0.5% per day in relation to Amoxicillin only in seawater. This suggests that Amoxicillin Trihydrate in close proximity to the delivery system in oceanic conditions is not negatively affected by the delivery system.



Graph 2: Study B Stability Results-This graph represents ~1.5% degradation of API/day in Batch 18006-B submerged in simulated seawater.

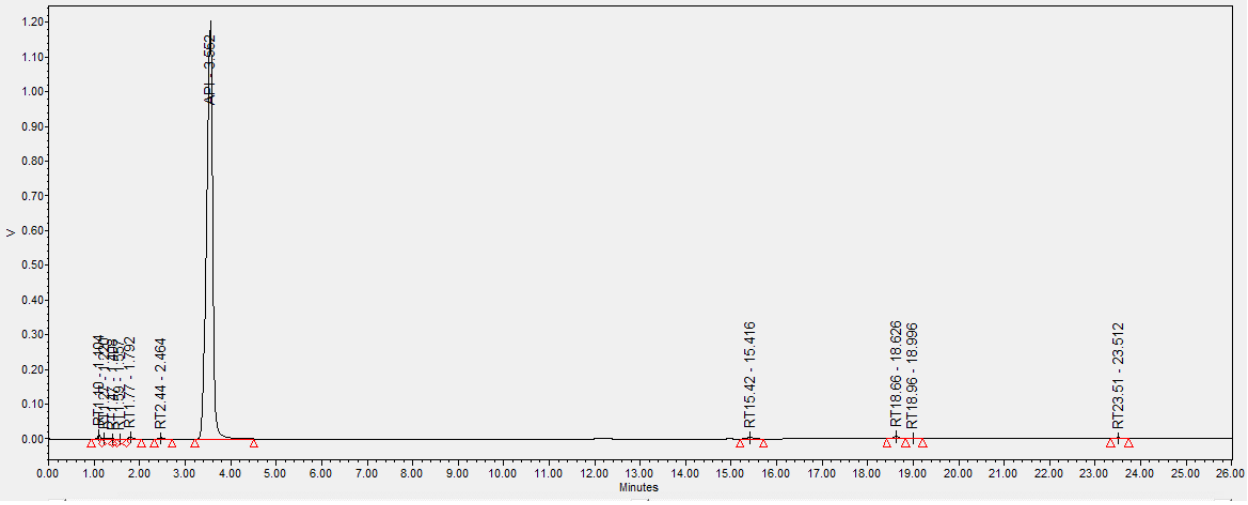


Image 3: Study B Initial Chromatogram-This graph represents the amount of API visible at 260nm in Batch 18006-B submerged in simulated seawater at the initial point of the stability study, with limited impurities.

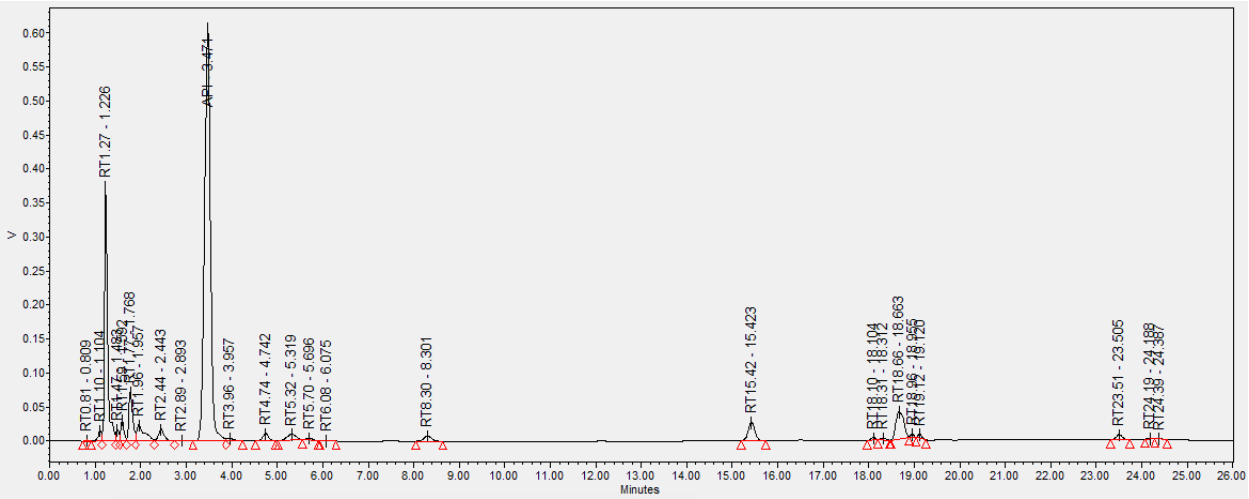
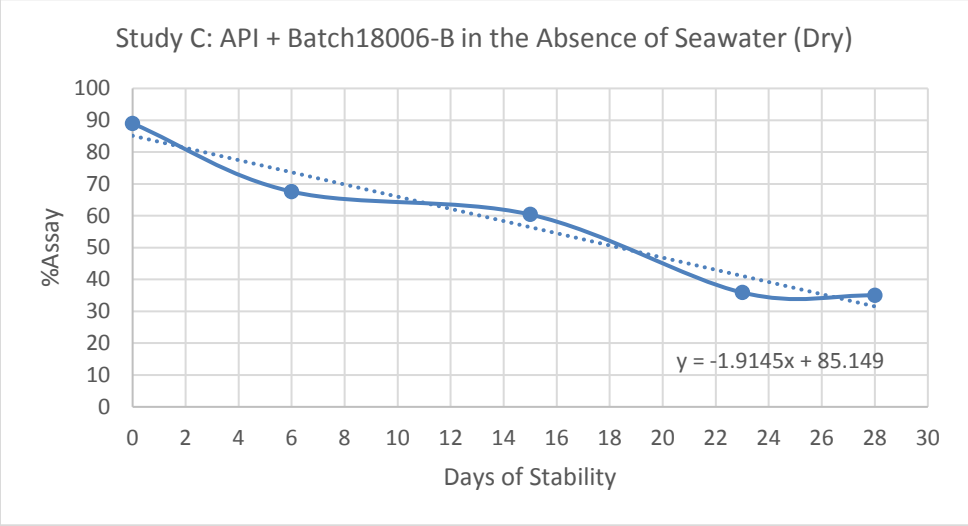


Image 4: Study B, 1 Month Stability Chromatogram- This graph represents the amount of API and all other visible impurities at 260nm that are produced during degradation of the API in Batch 18006-B submerged in simulated seawater at the end of 1 month.

Study C- Amoxicillin Trihydrate & Base 18006-B in the Absence of Seawater

Study C monitored the stability of Amoxicillin Trihydrate in the presence of the drug delivery system (Batch#137-18006-B) in 'dry' conditions (in the absence of seawater). This study was conducted to gain knowledge with respect to how long in advance the drug may be mixed into the placebo ointment before time of use. Samples saw a known quantity of Amoxicillin Trihydrate spread across the surface of a flattened disc of placebo drug delivery matrix. The samples were again quantified by HPLC assay methods. The degradation results were consistent with the degradation results in Study A and Study B at approximately 2% degradation per day. Unfortunately, issues involving the drug becoming matrix-bound appear to have negatively affected the study. No sample achieved an assay value above 89% including the T=0 assay. Peaks associated with known degradants were absent in concentrations that would match with the amounts of Amoxicillin absent from the results. This suggests that degradation may not have been to blame for the low results. Further studies regarding sample preparation will be needed before conclusions may be drawn in respect to this sub-study.



Graph 3: Study C Stability Results-This graph demonstrates ~2% degradation of API/day in Batch 18006-C in the absence of seawater but with a noted 90% initial recovery of the API.

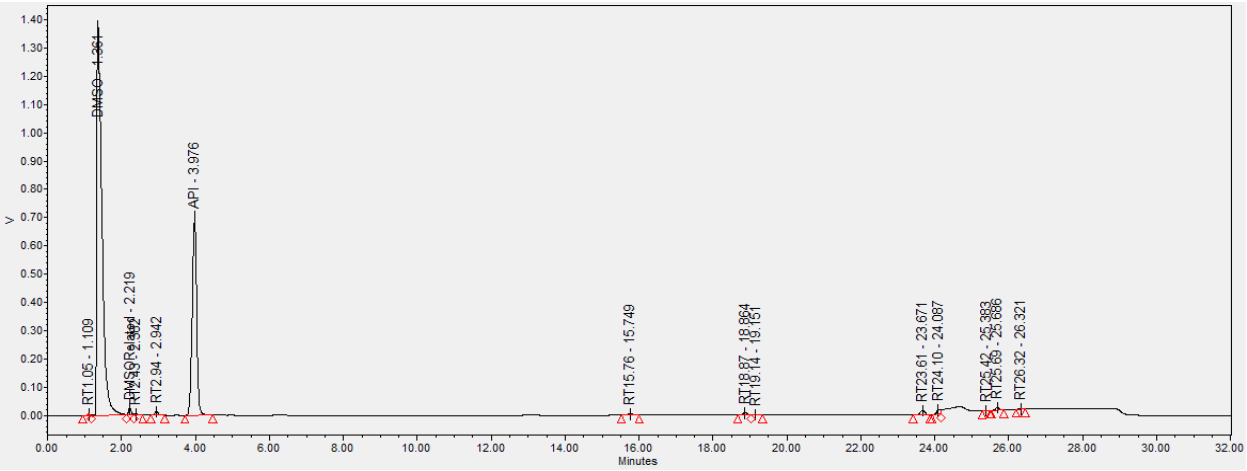


Image 5: Study C, Initial Chromatogram- This graph represents the amount of API visible at 260nm in Batch 18006-B at the initial point of the stability study, with limited impurities.

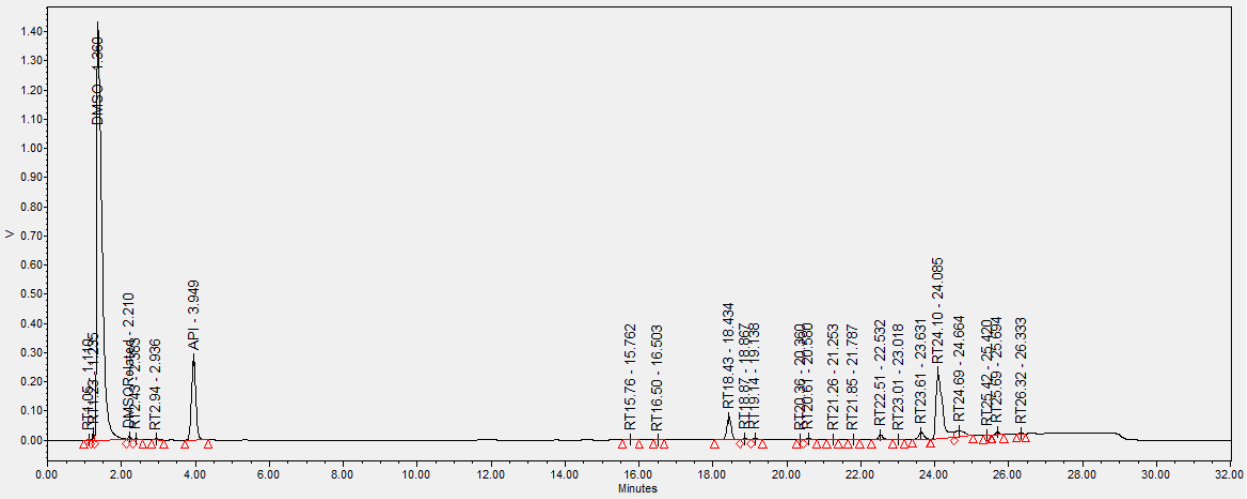


Image 6: Study C, 1 Month Stability Chromatogram- This graph represents the amount of API and all other visible impurities at 260nm that are produced during degradation of the API in Batch 18006-B at the end of 1 month.

Methods:

Study A Methods:

Amoxicillin Trihydrate in seawater samples were prepared by adding 31.25mg of Amoxicillin Trihydrate to a 25ml volumetric flask. (Exact weights of Amoxicillin for each sample were printed and later used to calculate exact concentrations per vial during HPLC analysis.) The flask was then filled with approximately 15ml of simulated seawater. The flasks were swirled multiple times by hand before a 5-minute period of sonication. At this point all API (active pharmaceutical ingredient-Amoxicillin Trihydrate) was sufficiently dissolved into the simulated seawater. Samples were then allowed to cool to room temperature. Once appropriately cooled the flasks were QS'd (Quantum Satis) with additional simulated seawater. Samples were dated, and left in a sealed, room temperature environment in the absence of UV light. A small 1.5ml aliquot was sampled for HPLC analysis the day of testing.

HPLC #	72	
Flow Rate	1.5mL/minute	
Injection Volume	10 µL	
Runtime	26 min	
Wavelength Monitored	260 nm	
Column Temperature	40°C	
Pump Mode	Gradient	
HPLC Column	Agilent Eclipse Plus C18 5um x 4.6mm x 150mm SN:USUXB12969	
Time	Mobile Phase A Concentration	Mobile Phase B Concentration
0	97	3
10	97	3
22	75	25
26	97	3

Table 1: Study A HPLC Parameter

Study B Methods:

Amoxicillin Trihydrate paired with Batch#137-18006-B in simulated seawater samples were prepared in a separate fashion to those previously mentioned. Samples were prepared by first weighing approximately 950mg of Batch#137-18006-B placebo into scintillation vials. This was followed by weighing and adding approximately 37.5mg of Amoxicillin Trihydrate to the scintillation vial already containing the placebo ointment. (Exact weights of Amoxicillin for each sample were printed and later used to calculate exact concentrations per vial during HPLC analysis.) Each vial then received 15ml of simulated seawater. Finally, a small stir bar was added to each vial which were then left to stir at a slow laminar flow until the day of testing. Stirring samples were capped and stored in the absence of UV light at room temperature. HPLC samples were prepared by filtering approximately 10ml of sample through a 0.2micron Nylon filter. Exactly 3ml of filtered sample was then added to a separate 10ml volumetric flask. Each flask was then QS's with more simulated seawater before a sub-sample was taken for HPLC analysis.

HPLC #	72	
Flow Rate	1.5mL/minute	
Injection Volume	10 µl	
Runtime	26 min	
Wavelength Monitored	260 nm	
Column Temperature	40°C	
Pump Mode	Gradient	
HPLC Column	Agilent Eclipse Plus C18 5um x 4.6mm x 150mm SN:USUXB12969	
Time	Mobile Phase A Concentration	Mobile Phase B Concentration
0	97	3
10	97	3
22	75	25
26	97	3

Table 2: Study A HPLC Parameters

Study C Methods:

Amoxicillin Trihydrate paired with 'dry' Batch#137-18006-B samples were prepared in a similar fashion to those previously mentioned. Samples were prepared by first weighing approximately 950mg of Batch#137-18006-B into scintillation vials. A micro spatula was then used to manipulate the placebo ointment into a thin disc which covered the bottom of the scintillation vial. This was followed by adding approximately 37.5mg of Amoxicillin Trihydrate to the vial. (Exact weights of Amoxicillin for each sample were printed and later used to calculate exact concentrations per vial during HPLC analysis.) Each vial was then rolled, tapped, and manipulated to allow the dry Amoxicillin to cover the surface of the placebo ointment disc. Samples were then capped and stored in the absence of UV light at room temperature until time of use. HPLC samples were prepared by adding 10ml of DMSO (Dimethyl-Sulfoxide) to each sample the day of HPLC analysis. Each sample was then vortexed for 2-minutes followed by 5-minutes of sonication followed by additional 2-minutes of vortex mixing. Each sample was then allowed to sit for 30-minutes. The liquid contents of each sample were then filtered through a 0.2micron nylon filter. Exactly 2mL of filtered sample was then added to a separate 10ml volumetric flask. Each flask was QS'd with more simulated seawater before a sub-sample was taken for HPLC analysis.

HPLC #	146	
Flow Rate	1.5mL/minute	
Injection Volume:	10 µl	
Runtime:	32 min	
Wavelength Monitored	260 nm	
Column Temp.	40°C	
Pump Mode	Gradient	
HPLC Column	Agilent Eclipse Plus C18 5um x 4.6mm x 150mm SN:USUXB12969	
Time	Mobile Phase A Concentration	Mobile Phase B Concentration
0.00	97	3
10.00	97	3
22.00	75	25
22.10	20	80
27.00	20	80
27.10	97	3
32.00	97	3

Table 3: Study A HPLC Parameters

Simulated Seawater	35.95g of instant ocean/L of deionized water/RO water
Mobile Phase A	2.72g/L of monobasic potassium phosphate to deionized/RO water. Adjust to pH 5.0 +/- 0.1 with Potassium Hydroxide or Phosphoric Acid
Mobile Phase B	100% HPLC Grade Methanol

Table 4: Reagents Common to All Testing

Conclusion:

The stability of Amoxicillin Trihydrate in seawater (Study A) exceeded expectations, maintaining at least 50% efficacy over the study period of 1 month. The degradation rate of the active ingredient into sea water proved to be less than 2% degradation per day. The stability of Amoxicillin Trihydrate suspended in coral ointment batch 18006-B while submerged in seawater (Study B) was consistent with Study A results. In Study C, not all Amoxicillin was recovered, this was likely due to exaction issues during sample preparations, however due to this error it cannot be conclusively stated that degradation of Amoxicillin Trihydrate did not occur when mixed with dry ointment (ointment not in the presence of seawater). Results of Study C were still positive with a 2% degradation consistent with Study A and Study B, however the lack of full recovery should be taken into consideration. Thus, is it recommended that Amoxicillin be added to the ointment as close to the application time as possible. These studies suggest that coral ointment batch 18006-B is a feasible delivery system for the Amoxicillin Trihydrate in a high salinity aqueous environment.