# Stability of Epinephrine in a 0.9% Saline Solution

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Spencer Wade, DDS

Graduate Program in Dentistry

The Ohio State University

2019

Thesis Committee:

Bryant Cornelius, Adviser

Courtney Jatana

Kelly Kennedy

Michelle Humeidan

## Abstract

Objective: By law, pharmaceutical companies clearly label their medications with an expiration date, but the debate behind the “new expiration date” that drugs are given after being diluted is ongoing. Some medications, including epinephrine, commonly come in concentrations which are unusable for clinical anesthesiology. Epinephrine is generally used during emergency situations, making it difficult to perform an urgent dilution amidst a declining clinical scenario. Emerging evidence indicates that not only do medications remain stable past their original pharmaceutical expiration date, but their stability remains intact even after dilution. The objective of this study was to determine the stability of epinephrine over time in a 10ml normal saline pre-filled syringe stored in typical perioperative environments.

Methods: All samples were prepared by diluting 1 mg/ml of epinephrine to 10mcg/ml by using normal saline pre-filled syringes. The samples were then left for 0, 15, 30, 60, and 90 day(s) prior to final analysis and stored in one of four different laboratory environments to emulate common clinical scenarios (light, dark, room temperature, and refrigerated). Capillary zonal electrophoresis was then used to measure the chemical degradation of epinephrine over time. Additionally, agar plating was used to measure bacterial growth in all samples.

Results: No significant differences were detected in levels of epinephrine between the 0, 15, 30-day and 0, 60, 90-day samples in each of the four environments. Additionally, bacterial growth was not detected in any of the samples.

Conclusions: Epinephrine remains stable in a normal saline pre-filled syringe for up to 90 days in common settings of lighting and temperature (*i.e.,* sample degradation does not exceed the sample variance attributable to sample dilution). Therefore, pre-diluting epinephrine for unanticipated clinical emergencies may remain clinically useful for up to 90 days, thus improving patient safety, access to medications, and overhead costs.

Acknowledgements

I am incredibly thankful for the opportunity that The Ohio State University has granted me over the last seven years. Every aspect of my education from dental school to residency has not only made me a more critical thinker and astute clinician, but also simply a better person.

Thank you to all the faculty members, students, and staff at The Ohio State University College of Dentistry for nurturing top-notch dentists through your rigorous predoctoral curriculum. I made lifelong friends and colleagues through an arduous four years and will continue the traditions of being a Buckeye.

Thank you to the past and current dental anesthesiology program directors who's only goal was to ensure that my co-residents and I received nothing more than an exceptional education. Drs. Simon Prior, Sarah Leach, and Bryant Cornelius, you have all been invaluable to shaping me into the person and clinician that I am today, and I will always be grateful.

Thank you to all oral and maxillofacial surgery faculty, residents, and assistants. You have all been incredible to work with and supportive in times where I struggled. It has been an absolute honor for you to instill trust in me to treat your patients. Best of luck to all your future endeavors.

Thank you to everyone part of University Hospital and Nationwide Children's Hospital. The combined efforts of students, residents, fellows, faculty, nurses, assistants, and anesthesia techs contributed only positive to my training. I will always feel privileged to

have been trained by, trained under, and trained beside you. It was an incredible journey to endure.

A noted thank you to Drs. Courtney Jatana, Kelly Kennedy, Michelle Humeidan and Bryant Cornelius. You have not only striven to give me the best possible education through your continuous efforts as faculty but have also gone the extra step in assisting me with my master's project. There have been a lot of questions, meetings and discussions, and I will always be thankful for your support and guidance.

Drs. Joshua Blakeslee, Hongrui Wang, Dan McKim, and Patrick Dib, thank you for all your contributions and using your expertise to help organize, setup and analyze this experiment. You are all gifted and fantastic researchers who have proven to be incredibly patient when dealing with someone who does not have a fraction of the research background you possess. I hope our next lineage of residents gets to work with you on their future projects.

Finally, Caroline Sawicki, she has gone above and beyond as both a clinician, researcher, and friend. I have been so blessed to have her in my life as she continues to push me to not only be a better researcher and professional but also a better person.

# Vita

April 2012 ..... Bachelor of Science – University of
…… Michigan, Ann Arbor, Michigan

April 2016 ..... Degree of Dental Surgery – College of
…… Dentistry, The Ohio State University,
…… Columbus, Ohio

June 2016 to Present ..... Certificate Maxillofacial Anesthesiology
…… Department of Anesthesiology
…… The Ohio State University

## Publications

1) **Spencer Wade DDS,** Peter Larsen, DDS, Bryant Cornelius, DDS, MPH. Case Report “Hemophilia A: Preparing For Outpatient Oral Surgery”, The Pulse 2019.2 28-30

2) **Spencer Wade DDS**, George Koutras, DDS, Bryant Cornelius, DDS, MBA, MPH. Case Report “Illicit Opioid Overdose in a University Dental Clinic”. The Pulse 2018.2 13-14.

3) Emam, H. A., C. A. Jatana, **S. Wade**, and D. Hamamoto. 2018. “Dental Student Self-Assessment of a Medical History Competency Developed by Oral and Maxillofacial Surgery Faculty.” *European Journal of Dental Education*. https://doi.org/10.1111/eje.12222

## Fields of Study

Major Field: Dentistry

Table of Contents

List of Tables

List of Figures

# Introduction

Epinephrine is one of the most commonly used emergency drugs and is rarely used during outpatient general anesthesia. In most clinical settings, epinephrine comes packaged in two forms: 1) a 1mg/1ml vial which must be diluted for clinical use and 2) a prepackaged 10ml syringe at 100ug/ml that is ready for IV use. During urgent situations, epinephrine at doses of 10ug/ml in common practice is used for mild refractory hypotension, bronchospasm, bradycardia, and allergic reactions. In emergency situations, 100ug/ml solutions are used for severe refractory hypotension, severe anaphylaxis, and advanced cardiac life support (ACLS).

In a private practice setting, it would be beneficial to the sole anesthesia provider to have low dose epinephrine on hand for urgent situations. In these settings, the anesthesia provider is typically the only one with the appropriate training and aptitude to handle the crisis. By holding onto these medications for longer than the standard 24 hours, patient safety would be improved in non-hospital based settings. Additionally, this would reduce time, cost, waste and resources by not having to prepare and discard epinephrine solutions as often.

Emerging evidence indicates that drugs remain stable past their labeled pharmaceutical expiration dates [1,2]. Despite this, no study to date has replicated a typical clinical office environment (i.e., private practice setting) for drug storage and analysis of epinephrine concertation and bacterial growth. Previous experimentation has shown that epinephrine can resist degradation, but there are not any studies to date investigating epinephrine's degradation when prepared at low dose in common peri-operative settings for longer

timepoints[3-8]. This project seeks to investigate epinephrine's stability and bacterial load in a 10ml normal saline syringe after a double dilution stored in standard in-office peri-operative conditions for up to 90 days.

## Methods

Sample Preparation:

The normal saline syringes [BD - Posiflush (Franklin Lakes, NJ)] come prepackaged with 10ml of sterile 0.9% saline. One milliliter of this solution was disposed prior to the dilution to achieve a total dilutional volume of 10ml once the 1ml of epinephrine sample was added.

1mg/1ml of epinephrine [Adrenalin - Epinephrine injection USP (Par Pharmaceutical, NY)] from the same lot were first diluted into the 9ml of normal saline yielding a concentration of 100mcg/ml. Then, 1ml of the previous 100mcg/ml sample was diluted again into a second 9ml saline syringe yielding a final dilution of 10mcg/ml.

Alcohol swabs [BD - Alcohol Swabs (Franklin Lakes, NJ)] were used to wipe the epinephrine vials prior to puncturing with a blunt tip needle [BD Blunt Fill Needle, 18 G x 1-1/2" (Franklin Lakes, NJ)]. For both dilutions, mixture was accomplished by flipping the syringes back and forth multiple times. Non-sterile gloves [Kimberly Clark – Purple Nitrile Gloves (Irving, TX)] and procedural mask [Halyard Health – Procedural Mask (Alpharetta, GA)] were also worn during the serial dilution.

Three samples were made total for each of the four environments (Light/No Light; 4˚C/20˚C) at our five time points (0, 15, 30, 60, 90 days) yielding 60 total samples. All double dilutions were performed by the same person for consistency.

Environments:

Four standard peri-operative environments were emulated with a mixture of lighting and temperature settings.

Three 10ug/ml solutions were placed into each of the following settings:

- 4 ˚C (Refrigerator) with fluorescent lighting
- 4 ˚C (Refrigerator) without fluorescent lighting
- 20 ˚C (Room Temperature) with fluorescent lighting
- 20 ˚C (Room Temperature) without fluorescent lighting

Samples were frozen after storage and kept at -20 ˚C until analysis by capillary zonal electrophoresis (CZE)

Capillary Zonal Electrophoresis:

Capillary zonal electrophoresis (CZE) was then used to measure the levels of epinephrine present in the samples. In these CZE studies, an electrical charge was used to separate epinephrine and related isomers based on the charge-to-mass ratios of the molecules. Epinephrine was detected and measured using its spectral absorbance (*i.e.,* ability to absorb specific wavelengths of light). Analysis of epinephrine standards confirmed that absorbance of light (measured in absorbance units, or A.U.) was directly proportional to the amount of epinephrine present in the samples across the range of concentrations used in this study. CZE measurements were performed using a Beckman-Coulter P/ACE MDQ unit at the OARDC Metabolite Analysis Cluster (OMAC).

Concentrations of epinephrine were determined using concentration curves generated using authentic epinephrine standards from the 1mg/ml epinephrine vial (stored at room temperature) of the same lot as the experimental group.

Sterility Testing:

Sterility testing was performed by streak-plating under aseptic conditions inside a BSL-2 biosafety cabinet. Disposable, pre-sterilized needles were used to streak plate approximately 0.1 mL of 10ug/ml of the epinephrine across agar plates [Fisher Scientific - Blood Agar (Waltham, MA)]. Agar plates were then transferred to a 37°C incubator for 48 hours. After 48 hours, the agar plates were analyzed for presence of microbial growth using NIH ImageJ v1.52a with the Colony Counter plugin. Software analysis revealed no microbial colony growth from any of the 60 epinephrine samples.

Statistical Analysis:

All data are expressed as averages ± standard deviation (SD). To determine significant main effects and interactions between main factors, data was analyzed using multi-factor ANOVA using RStudio (version 1.1.456). *Post hoc* analyses are graphically presented as figures. Threshold for statistical significance was set to $p<0.05$.

## Results

Figure 1 and Table 1: Samples for both the 0, 60, 90-day trial and the 0, 15, 30-day trial were both created by double dilution technique to yield their projected 10mcg/cc concentrations. All samples were stored in environments, either (dark/4ºC, dark/20ºC, light/4ºC, light/20ºC) for either 60 or 90 days. CZE was then used to quantify epinephrine in all conditions (Figure 1). There were no significant differences in the absorption of epinephrine between any of the tested environments ($p>0.05$). Epinephrine levels are represented in absorbance units (A.U.); bars represent average ± std. deviation. Table 1 represents raw values (average and standard deviation) for epinephrine absorption.

Figure 2 and Table 2: Samples for both the 0, 60, 90-day trial and the 0, 15, 30-day trial were both created by double dilution technique to yield their projected 10mcg/cc concentrations. All samples were stored in environments, either (dark/4ºC, dark/20ºC, light/4ºC, light/20ºC) for either 60 or 90 days. CZE was then used to quantify epinephrine in all conditions (Figure 2). There were no significant differences in the concentration of epinephrine between any of the tested environments ($p>0.05$). Epinephrine levels are represented in mcg; bars represent average ± std. deviation. Table 2 represents raw values (average and standard deviation) for epinephrine concentration in mcg.

Figure 3 and Table 4: Samples for both the 0, 60, 90-day trial and the 0, 15, 30-day trial were both created by double dilution technique to yield their projected 10mcg/cc concentrations. All samples were stored in environments, either (dark/4ºC, dark/20ºC, light/4ºC, light/20ºC) for either 15 or 30 days. CZE was then used to quantify epinephrine in all conditions (Figure 3). There were no significant differences in the absorption of epinephrine between any of the tested environments ($p>0.05$). Epinephrine levels are

represented in absorbance units (A.U.); bars represent average ± std. deviation. Table 4 represents raw values (average and standard deviation) for epinephrine absorption.

Figure 4 and Table 5: Samples for both the 0, 60, 90-day trial and the 0, 15, 30-day trial were both created by double dilution technique to yield their projected 10mcg/cc concentrations. All samples were stored in environments, either (dark/4ºC, dark/20ºC, light/4ºC, light/20ºC) for either 15 or 30 days. CZE was then used to quantify epinephrine in all conditions (Figure 4). There were no significant differences in the concentration of epinephrine between any of the tested environments ($p>0.05$). Epinephrine levels are represented in mcg; bars represent average ± std. deviation. Table 5 represents raw values (average and standard deviation) for epinephrine concentration in mcg.

Table 3: There is no significant difference in epinephrine absorption or concentration between 0, 60, and 90 day(s) regardless of day, light or temperature conditions when compared independently ($p>0.05$). There is no significant interaction between day and light condition ($p>0.05$). However, there is a significant interaction between day and temperature ($p = 0.471$). There is no significant interaction between light and temperature conditions ($p>0.05$). There is no significant interaction between day, light and temperature conditions ($p>0.05$).

Table 6: There is no significant difference in epinephrine absorption or concentration between 0,15, and 30 day(s) regardless of day, light or temperature conditions when compared independently ($p>0.05$). There is no significant interaction between day and light conditions ($p>0.05$). There is no significant interaction between day and temperature conditions. There is no significant interaction between light and temperature conditions ($p>0.05$). There is no significant interaction between day, light and temperature conditions ($p>0.05$).

Figure 5 and Table 7: There is not any bacterial growth detected in all of the sixty experimental samples. There is no bacterial growth detected in the negative control. There is bacterial growth detected in positive control. There is bacterial growth detected in positive control and contaminated sample 58x, 400 and 450 CFU, respectively.

Figure 6: Relationship between absorption units (A.U.) and epinephrine concentration in CZE analysis.

## Discussion

Statistical analyses (ANOVA) showed no significant effect of light, temperature, or storage time on epinephrine levels over the 90 days (*i.e.,* changes in epinephrine level resulting from light, temperature, or storage time were less than those resulting from sample-to-sample variability). These data support a relatively high stability for epinephrine in solution, with minimal degradation observed across all standard storage environments regardless of the conditions tested.

A significant interaction was observed between day and temperature for our 0, 60, 90-day trial. This was interesting, as no other significant interactions between day and temperature were observed in previous or subsequent experimental runs. Close observation of the individual data points (Table 2) shows a large sample-to-sample variation, specifically a low outlier of 6.6mcg/ml (60-day/20 ˚C/light) with the next lowest being 8.0mcg/ml. Interestingly, concentrations of epinephrine this low were not detected in 90-day samples (which were all higher), indicating that this low concentration outlier is more likely the result of dilution variance rather than degradation. Therefore, while we cannot rule out the significance here, this result must be interpreted extremely cautiously, and we have concluded that the interaction observed is likely the result of potential sample-to-sample variance.

As previously mentioned, precision instrumentation for dilution was not used as the goal was to reflect a practical clinical application. Samples for analyses were prepared using gross approximation with pre-filled saline syringes, blunt tip needles, and highly concentrated epinephrine (1:1000). The expected concentration after this double dilution

is 10ug/ml but our final concentration ranged from 6.6 to 18.7mcg/ml (Table 2, Table 5). This is likely due to the imprecise and inaccurate instrumentation (specifically, the use of syringes and blunt tip needles rather than analytical pipettes) used during a double dilution procedure, leading to decreased precision and accuracy among samples in the same timepoint and environment. This error would be multiplied during sample preparation, as each sample required two uses of the pre-filled syringes: once to draw the initial sample of epinephrine from the vial, and again to pull 1ml of sample from the 100mcg/ml pre-filled syringe to complete the final dilution. While variance in sample preparation is the most plausible explanation for the observed sample-to-sample variance, an alternative hypothesis for the observed range of concentrations is that storing samples at -20 ºC may have induced some degradation in samples prior to analysis. However, this is an unlikely explanation, as it would result in a consistent under-representation of concentrations in samples with across-the-board reductions in peak area, rather than the stochastic variance observed across samples at all time points.

Finally, observed variability in the samples may have resulted from variance presence in the epinephrine solutions used to generate samples. Medications found in the vial prior to dilution may not have equivalent concentrations, as United States Pharmacopeia manufacturer standards must contain 90-115% of the noted product concentration. This could account for an additional increase in sample intra-variability as any sample could possibly have 0.90mg to 1.150mg in a 1ml vial of epinephrine. However, this variance would completely explain that observed in the samples, as our range of 6.6 to 18.7mcg/ml extends above and below the theoretical double dilution range of 9.0 to 11.50mcg/ml. Interestingly, Beasley et al. showed similar results, and found that the experimentation phase had higher concentrations than those present in control

samples[9]. Regardless, this leads to a popular mantra in the clinical world we live by the phrase “Titrate to effect”.

Drug degradation resistance has been investigated in-depth to preserve valuable resources. The use of brown UV-protecting bags and tinted vials has been used on epinephrine and other drugs to resist light degradation[10]. Chemical interactions can influence epinephrine degradation as well. Bicarbonate has been shown to cause more rapid epinephrine degradation[11]. However, adding bisulfite has been shown to enhance its stability especially with direct heat exposure[12]. Regardless, special precautions to epinephrine stability by chemicals or barriers were not used in this study to give an accurate reflection of a practical non-hospital-based environment.

Epinephrine degradation can remain relatively stable even in non-ideal storage conditions. Kerddonfak et al found that high concentration epinephrine drawn up in syringes carried by individuals with known anaphylaxis in settings of elevated temperatures and humidity, remained relatively stable after three months[13]. Interestingly, when epinephrine is stored in high humidity environments, its concentration remains more stable than when exposed to a drier environment[14]. Epinephrine seems to be more sensitive to degradation when undergoing cyclic elevated temperature changes but does appear to be resistant to repeated freeze-thaw cycles[9,12]. However, the previously mentioned studies do not represent standard peri-operative settings but do contribute to the notion that epinephrine remains stable in non-fluctuating environments, which this study’s observations reflect as well.

The agar plates did not exhibit any microbial growth. Besides diluted drugs not being as stable, the other common notion taught in hospitals is that they become contaminated overtime when not stored in their original container thus unacceptable for clinical use. As previously mentioned, the standard aseptic technique adhered to represents clinical

anesthesia as only a mask, non-sterile gloves, and an alcohol swab to sterilize the silicone tops prior to puncturing were utilized. Like all living species, bacteria needs a glucose, fat, and protein source to survive, these are not available in a normal saline and epinephrine solution unless exogenously supplied.

The nature of CZE make it difficult, if not impossible, to directly compare the concentrations and absorptions of the 0, 15, 30-day trial to the 0, 60, 90-day trial. This is because batches of samples were measured in overlapping batches on different capillaries; and epinephrine peaks exhibited retention times and concentration-response kinetics specific to each capillary. Changing capillaries between batches is necessary, as the large voltages used in CZE-based separations result in peak "drift" over time, making it impossible to run the large numbers of samples in the data set in one large single batch. The need to run samples in batches, as well as time needed for method optimization and to perform CZE system maintenance between batch runs resulted in individual samples being maintained at -20ºC for a time period ranging from five to six months. However, as previously mentioned, epinephrine remains stable after multiple freeze thaw cycles and in this instance, samples were subjected to only a single freeze-thaw event[9]. The overall effect is therefore likely minimal, especially as all the sample batches were frozen together with their respective 0-day controls.

There are multiple ways that the experiment could be further expanded upon. One of the study's limitations is that only epinephrine quantity and not quality are observed. Animal model supplementation and observing expected increases in cardiac output after administration would be excellent to investigate clinical effect preservation. This could be further extrapolated to investigate if a significant biologic difference (i.e., heart rate increases, blood pressure increases and duration of action) appears between our range (6.6-18.7mcg/ml) of epinephrine concentrations. Another consideration would be to

cultivate a cell model with an epinephrine-binding receptor which would activate or fluoresce in its presence. Another limitation of this study was the presence of endotoxins which were not tested. Even though bacteria did not grow on the blood agar medium which is conducive for growth, this does not mean that bacteria could not have been present at one time and left endotoxins from their cellular remains. This is another instance where an animal model would be beneficial as one could look for signs of infection and sepsis after medication use. These future implementations would reveal if there was an effect in epinephrine's biologic response or poses a health risk after storage.

One does not always have access to a pharmacy or pharmacy grade equipment. It is important to highlight how these samples were prepared under practical clinical conditions. This study reflects the sole anesthesia practitioner in a private practice or hospital environment where delaying bedside clinical treatment may increase morbidity or mortality. Regardless of the less-than-ideal storage conditions, it did not appear to have a significant impact on overall degradation nor contain actively replicating bacteria.

## Conclusion

Epinephrine concentrations exhibited no significant changes in pre-filled 0.9% saline syringe for at least 90 days in common clinical settings of lighting and temperature. Changes in epinephrine level resulting from light, temperature, or storage time were less than those resulting from sample-to-sample variability. In addition, the samples did not exhibit any bacterial growth on agar plates. Therefore, pre-diluting epinephrine for unanticipated clinical emergencies may remain clinically useful for up to 90 days. However, further studies should be warranted to determine that the samples do not contain bacterial remnants which may pose a hazard to human health.

# Appendix A: List of Figures

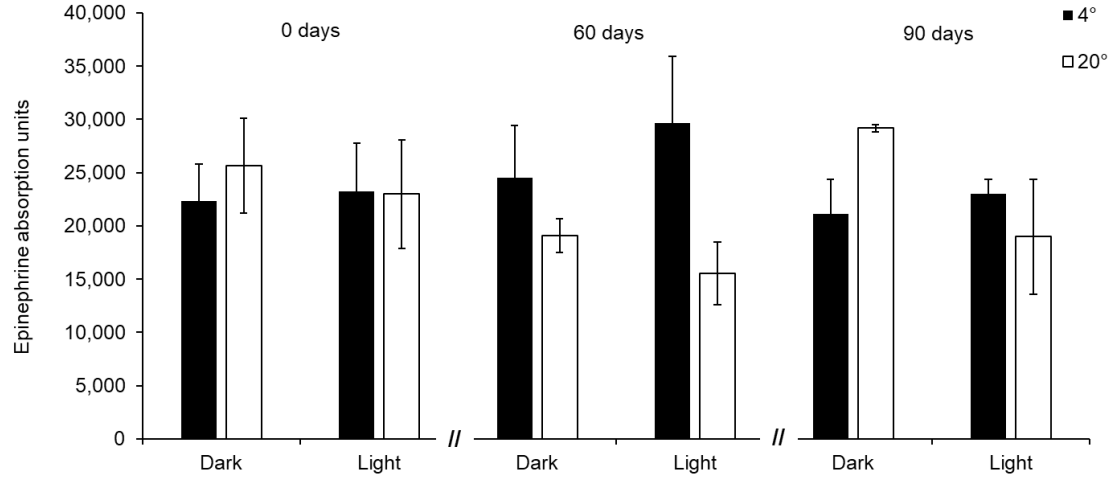


Figure 1: Absorption vs Environment (0,60, & 90 Days)

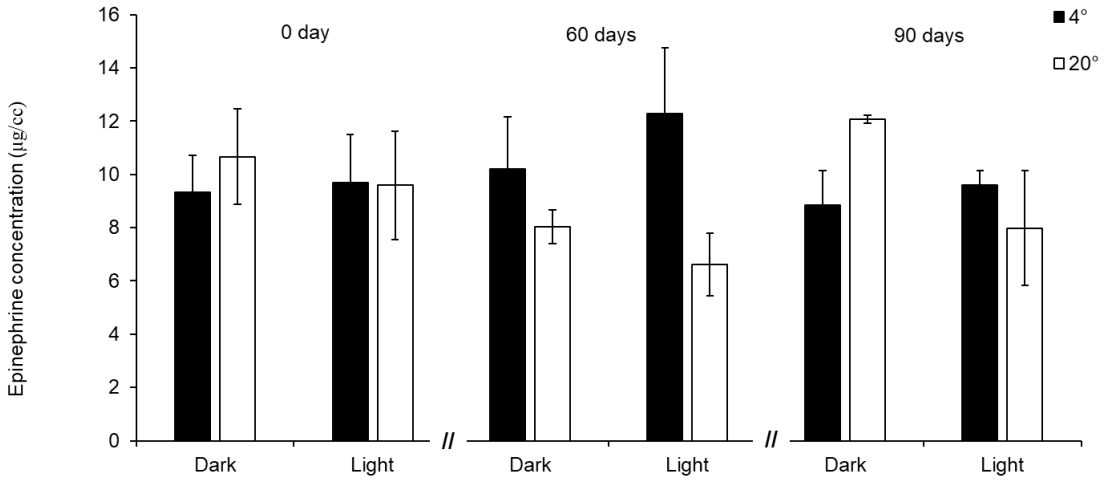


Figure 2: Concentration vs Environment (0, 60 & 90 Days)

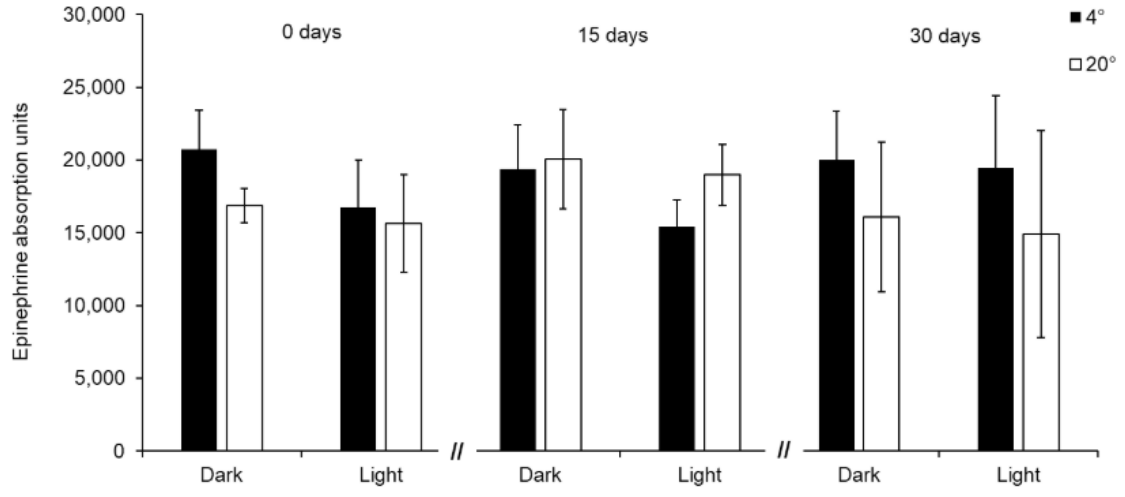


Figure 3: Absorption vs Environment (0, 15 & 30 days)

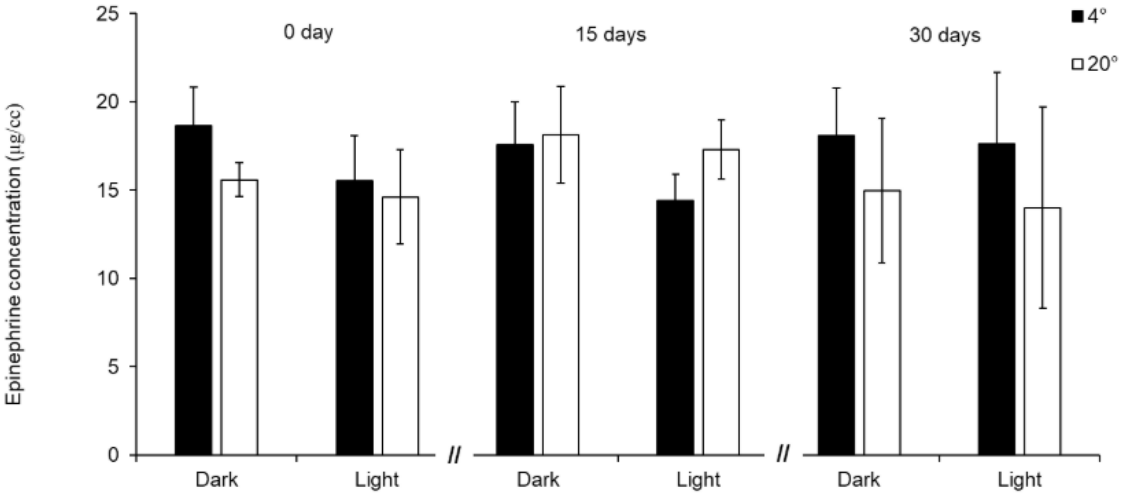


Figure 4: Concentration vs Environment (0, 15 & 30 Days)

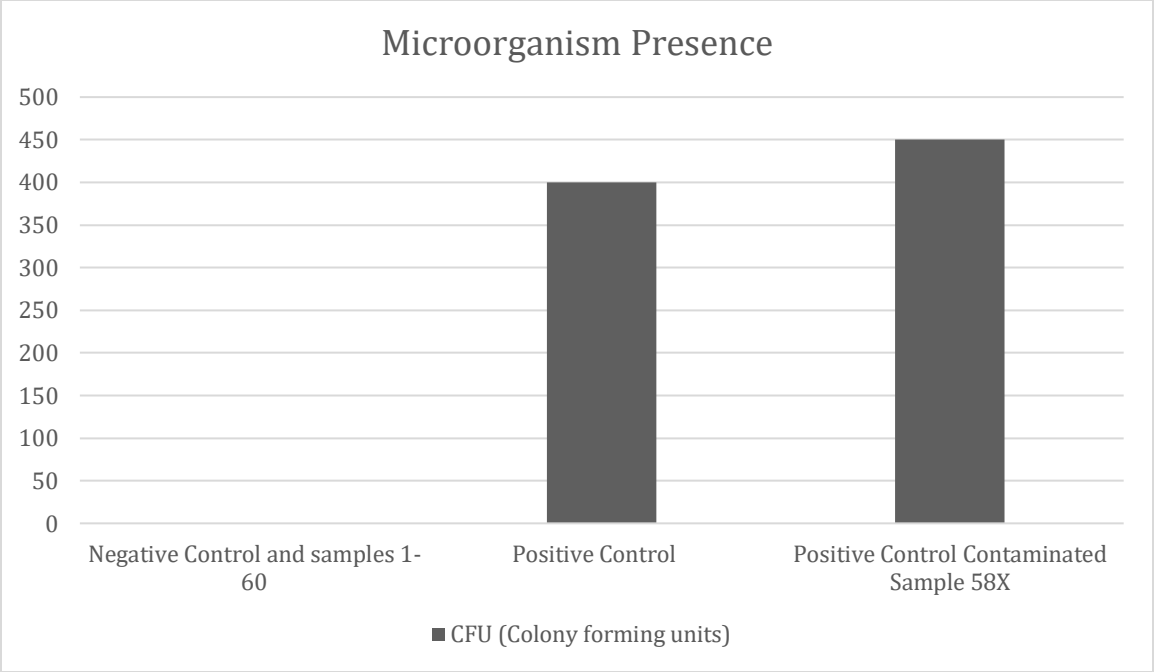


Figure 5: Samples vs Microorganism Presence

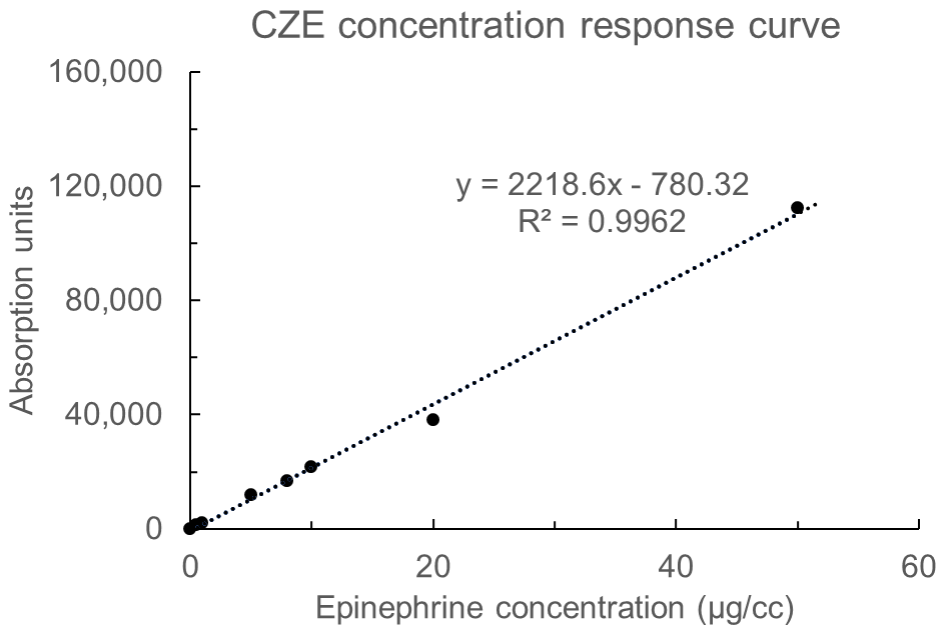


Figure 6: Absorption vs Concentration

## Appendix B: List of Tables

| AU (Peak area) Table | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day | 0 | | 60 | | 90 | |
| | Light condition | Dark | Light | Dark | Light | Dark | Light |
| Mean | 4° | 22339 | 23237 | 24548 | 29682 | 21138 | 22983 |
| | 20° | 25670 | 22994 | 19109 | 15549 | 29191 | 18977 |
| STD | 4° | 3448 | 4532 | 4872 | 6245 | 3207 | 1413 |
| | 20° | 4468 | 5078 | 1597 | 2944 | 345 | 5409 |

Table 1: Absorption vs Environment (0, 60 & 90 Days)

| Concentration Table (mcg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day | 0 | | 60 | | 90 | |
| | Light condition | Dark | Light | Dark | Light | Dark | Light |
| Mean | 4° | 9.3 | 9.7 | 10.2 | 12.3 | 8.9 | 9.6 |
| | 20° | 10.7 | 9.6 | 8.0 | 6.6 | 12.1 | 8.0 |
| STD | 4° | 1.4 | 1.8 | 1.9 | 2.5 | 1.3 | 0.6 |
| | 20° | 1.8 | 2.0 | 0.6 | 1.2 | 0.1 | 2.2 |

Table 2: Concentration vs Environment (0, 60 & 90 Days)

| | Day | Light | Temperature | Day Light | Day Temperature | Light Temperature | Day Light Temperature |
|---|---|---|---|---|---|---|---|
| *P* value | 0.65296 | 0.34142 | 0.13797 | 0.11953 | 0.00471 ** | 0.1416 | 0.48388 |

Table 3: Statistical Significance (0, 60 & 90 Days)

| AU (Peak Area) Table | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day | 0 | | 15 | | 30 | |
| | Light condition | Dark | Light | Dark | Light | Dark | Light |
| Mean | 4° | 20700 | 16777 | 19358 | 15408 | 19971 | 19414 |
| | 20° | 16862 | 15638 | 20051 | 18983 | 16083 | 14894 |
| STD | 4° | 2737 | 3200 | 3024 | 1853 | 3363 | 5023 |
| | 20° | 1175 | 3355 | 3425 | 2104 | 5128 | 7099 |

Table 4: Absorption vs Environment (0, 15 & 30 Days)

| Concentration Table (mcg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day | 0 | | 15 | | 30 | |
| | Light condition | Dark | Light | Dark | Light | Dark | Light |
| mean | 4° | 18.7 | 15.5 | 17.6 | 14.4 | 18.1 | 17.6 |
| | 20° | 15.6 | 14.6 | 18.1 | 17.3 | 15.0 | 14.0 |
| STD | 4° | 2.2 | 2.6 | 2.4 | 1.5 | 2.7 | 4.0 |
| | 20° | 0.9 | 2.7 | 2.7 | 1.7 | 4.1 | 5.7 |

Table 5: Concentration vs Environment (0, 15 & 30 Days)

| | Day | Light | Temperature | Day Light | Day Temperature | Light Temperature | Day Light Temperature |
|---|---|---|---|---|---|---|---|
| *P* value | 0.867 | 0.146 | 0.33 | 0.844 | 0.119 | 0.522 | 0.879 |

Table 6: Statistical Significance (0, 15 & 30 Days)

| Samples | Microorganism Presence / CFU |
|---|---|
| Negative Control, 1-60 | Negative |
| Positive Control | Positive / 400 CFU |
| Positive Control Contaminated Sample 58X | Positive/ 450 CFU |

Table 7: Samples vs Microorganism Presence

# References


1. Simons, F.estelle R., et al. “Outdated EpiPen and EpiPen Jr Autoinjectors: Past Their Prime?”*Journal of Allergy and Clinical Immunology*, vol. 105, no. 5, 2000, pp. 1025–1030., doi:10.1067/mai.2000.106042.
2. Lyon, Robbe C, et al. “Stability Profiles of Drug Products Extended beyond Labeled Expiration Dates.” *Journal of Pharmaceutical Sciences*, U.S. National Library of Medicine, July 2006, www.ncbi.nlm.nih.gov/pubmed/16721796.
3. Hoellein, L. and Holzgrabe, U. (2012). Ficts and facts of epinephrine and norepinephrine stability in injectable solutions. *International Journal of Pharmaceutics*, 434(1-2), pp.468-480.
4. Walker, Scott E, et al. “Stability of Norepinephrine Solutions in Normal Saline and 5% Dextrose in Water.” *The Canadian Journal of Hospital Pharmacy*, vol. 63, no. 2, 2010, doi:10.4212/cjhp.v63i2.896.
5. Ghanayem, N., Yee, L., Nelson, T., Wong, S., Gordon, J., Marcdante, K. and Rice, T. (2001). Stability of dopamine and epinephrine solutions up to 84 hours. *Pediatric Critical Care Medicine*, 2(4), pp.315-317.
6. Tremblay, M., Lessard, M., Trépanier, C., Nicole, P., Nadeau, L. and Turcotte, G. (2008). Stability of norepinephrine infusions prepared in dextrose and normal saline solutions. *Canadian Journal of Anesthesia/Journal canadien d'anesthésie*, 55(3), pp.163-167.
7. Heeb, R., Stollhof, B., Reichhold, J., Thiesen, J. and Krämer, I. (2017). Stability of Ready-to-Administer and Ready-to-Use Epinephrine and Norepinephrine Injection Solutions. *Pharmaceutical Technology in Hospital Pharmacy*, 2(4).
8. Carr, Roxane R, et al. “Stability of Epinephrine at Standard Concentrations.” *The Canadian Journal of Hospital Pharmacy*, vol. 67, no. 3, 2014, doi:10.4212/cjhp.v67i3.1356.
9. Beasley, H., Ng, P., McIntosh, S., Wheeler, A. and Smith, W. (2015). The Impact of Freeze-Thaw Cycles on Epinephrine. *Wilderness & Environmental Medicine*, 26(1), p.94.
10. Van Matre, E., Ho, K., Lyda, C., Fullmer, B., Oldland, A. and Kiser, T. (2017). Extended Stability of Epinephrine Hydrochloride Injection in Polyvinyl Chloride Bags Stored in Amber Ultraviolet Light–Blocking Bags. *Hospital Pharmacy*, 52(8), pp.570-573.
11. Robinson, J., Fernando, R., Sun Wai, W. and Reynolds, F. (2000). Chemical stability of bupivacaine, lidocaine and epinephrine in pH-adjusted solutions. *Anaesthesia*, 55(9), pp.853-858.
12. Church, W., Hu, S. and Henry, A. (1994). Thermal degradation of injectable epinephrine. *The American Journal of Emergency Medicine*, 12(3), pp.306-309.
13. Kerddofonfak, S., Manuyakorn, W., Kamchaisatian, w., Sasiskulporn, C., Teawosomboonkit, W., Benjaponpitak, S. (2010). The Stability and Sterility of Epinephrine Prefilled Syringe. *Asian Pacific Journal of Allergy and Immunology, vol28, pp. 53-57*
14. Rawas-Qalaji, M., Simons, F., Collins, D. and Simons, K. (2009). Long-term stability of epinephrine dispensed in unsealed syringes for the first-aid treatment of anaphylaxis. *Annals of Allergy, Asthma & Immunology*, 102(6), pp.500-503.