

## Background

- Acetaminophen is a common antipyretic commonly found in Tylenol.
- Acetaminophen metabolizes in the body once taken, but up to 9% can be excreted through urine. This will enter the water system, and into the environment.
- Small concentrations of acetaminophen can interfere with embryonic development, growth, behavior and more in fish populations.
- Bleach will be used to eliminate acetaminophen from water samples, acting like bleach tablets in toilet bowls.
- HPLC was utilized to measure acetaminophen concentrations in known samples.
- Kinetics experiments will be performed to find degradation over time.

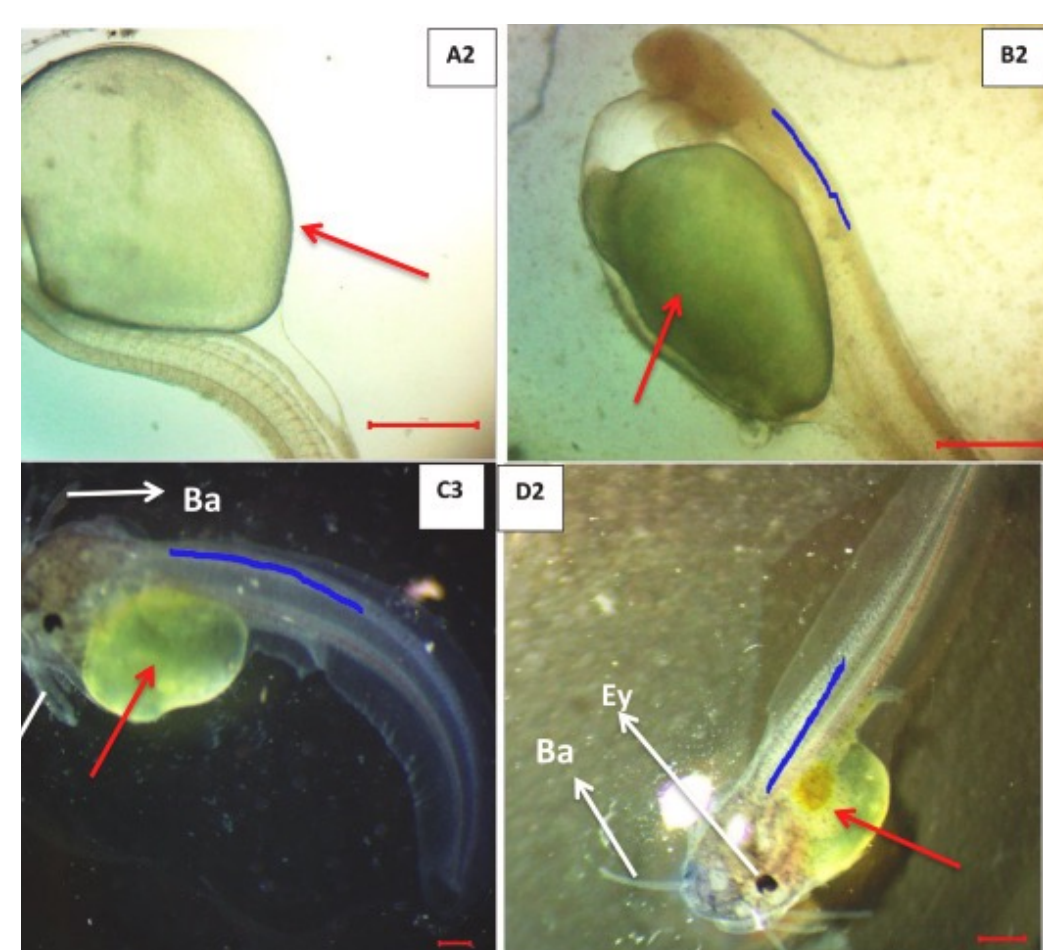


Figure 1: Pharmaceutical induced masses on fish.<sup>4</sup>

## Objectives

- Due to the commonness of acetaminophen, the need to eliminate it before it reaches wastewater is important.
- The goal of this research is to degrade acetaminophen in a water sample using bleach tablets and asses using HPLC.



Figure 2: Tylenol sales are more than \$300 million a year.

## Methods

- UV-Vis Spectroscopy: An acetaminophen solution of 10 ppm was made, and a UV-Vis spectrum was created.
- The  $\lambda_{max}$  of this solution was 242.5 nm.
- This was used in the UV detector for HPLC.

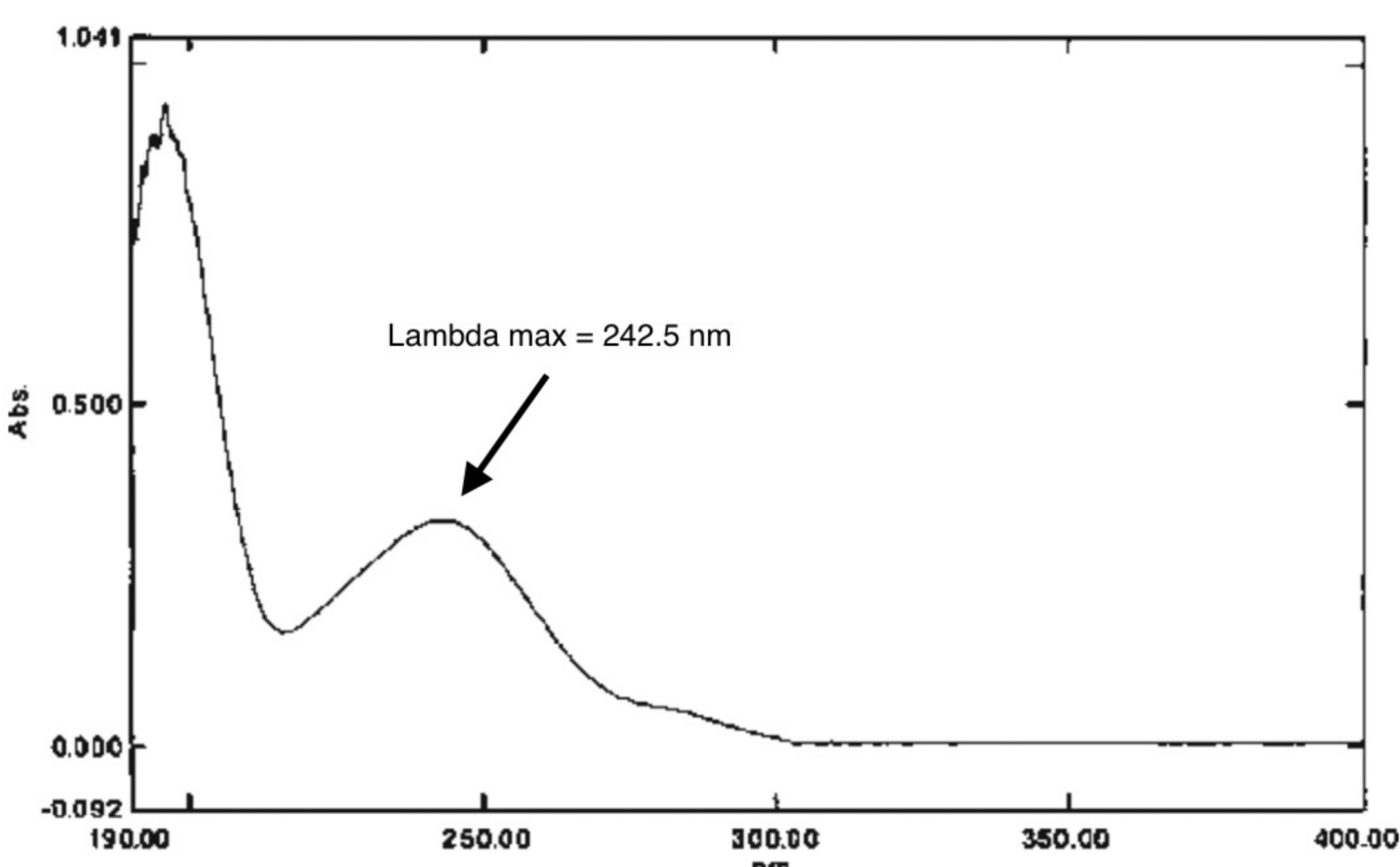


Figure 3: UV-Vis spectrum of 10 ppm acetaminophen. The  $\lambda_{max}$  is 242.5 nm.

## HPLC Method Development

- Standards from 5-10 ppm were measured.
- Initially a mobile phase of 94.5:5.5 water:acetonitrile composition was used with a flow rate of 1.5 mL/min in HPLC.
- No clear peaks produced, therefore glacial acetic acid and triethylamine was added to the HPLC grade water. The mobile phase was 94.1:5.5:0.2:0.2 water:acetonitrile:glacial acetic acid:triethylamine. Differences in viscosity required the water, glacial acetic acid, and triethylamine to be combined in the same container.
- The mobile phase was finalized by adding more water to decrease the retention time. The final mobile phase was 97.6:2:0.2:0.2 water:acetonitrile:glacial acetic acid:triethylamine. The new retention time was ~ 4.175 minutes.
- Standard solutions of 1, 2, 3, and 4 ppm were made from the 10 and 5 ppm solutions to create a calibration curve to track acetaminophen concentration.

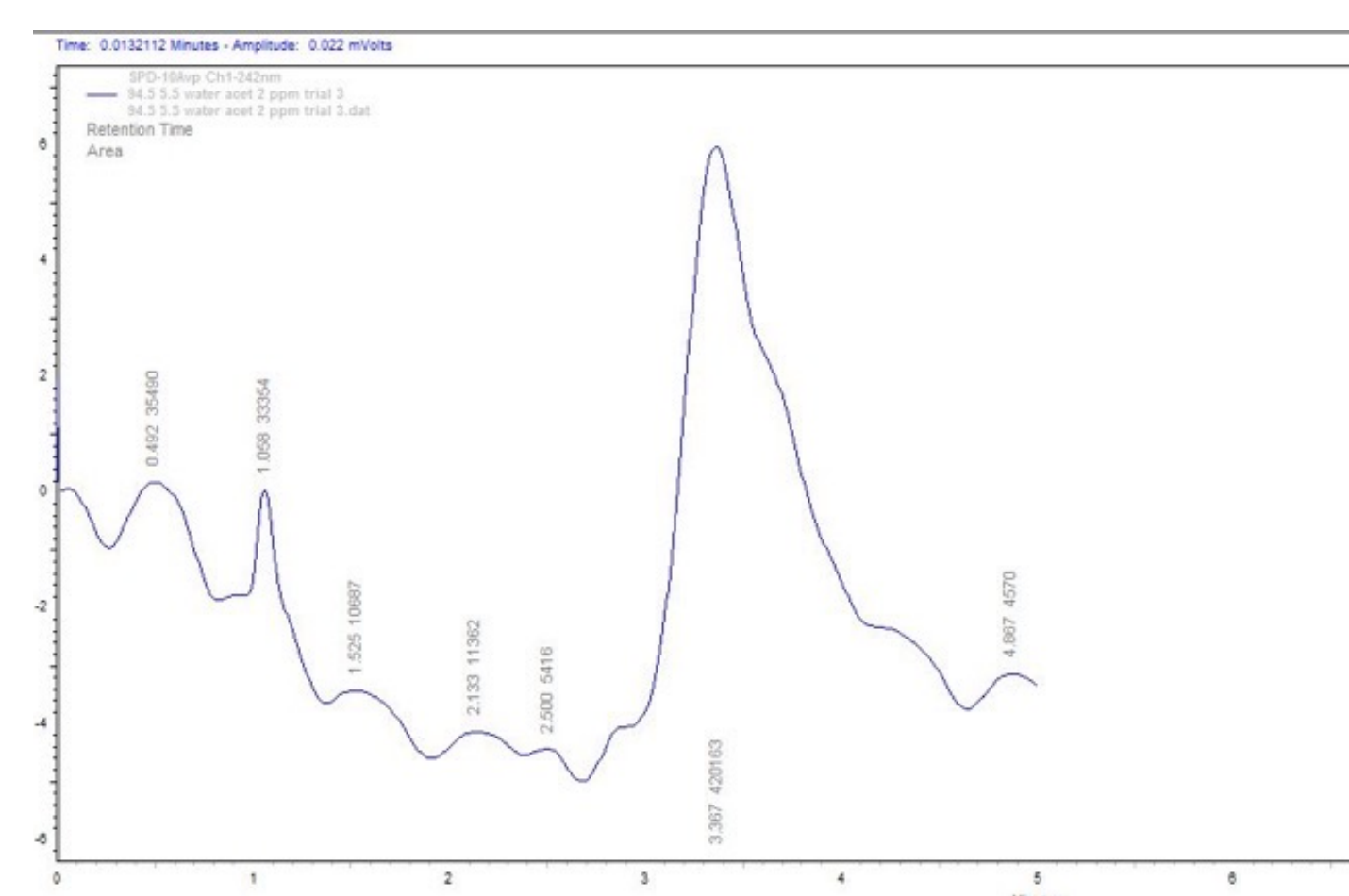


Figure 4: HPLC Chromatogram of 2 ppm Acetaminophen Solution. 94.5:5.5 water:acetonitrile in Separate Containers.

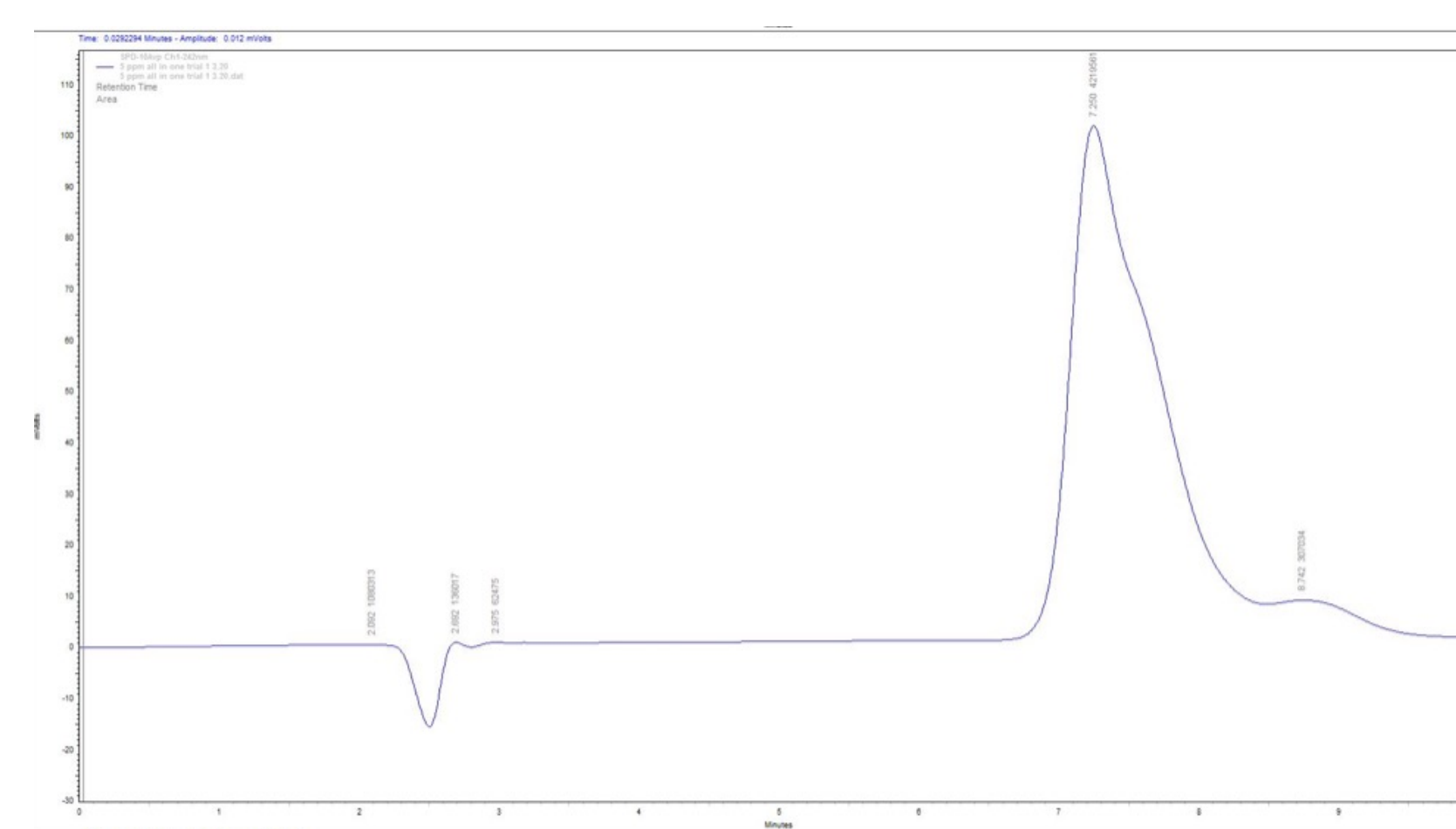


Figure 5: HPLC chromatogram of 5 ppm acetaminophen solution. 94.1:5.5:0.2:0.2 water:acetonitrile:glacial acetic acid:triethylamine in one container. Retention time of acetaminophen is 7.250 minutes.

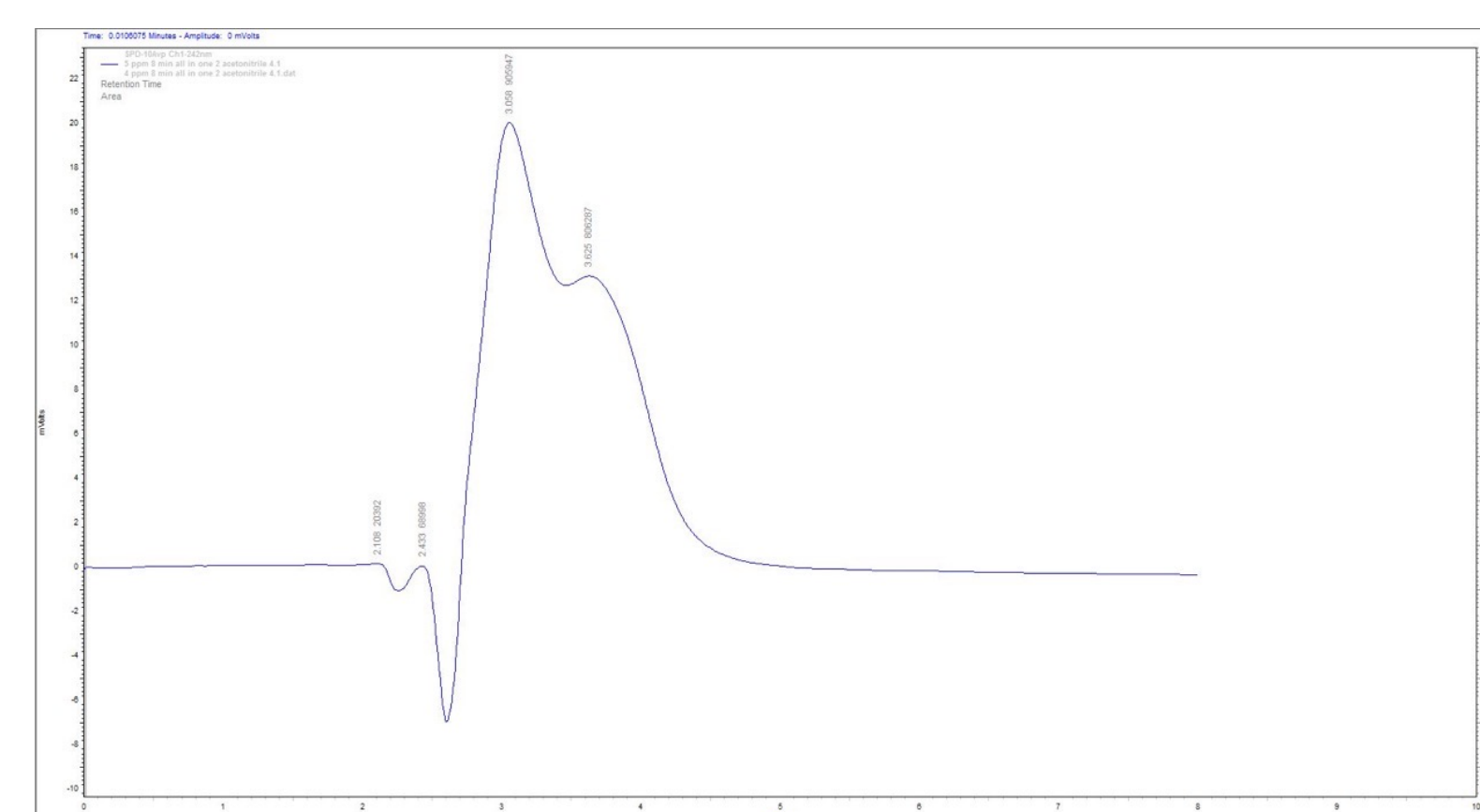


Figure 6: HPLC chromatogram of 5 ppm acetaminophen solution. Mobile phase is 97.6:2:0.2:0.2 water:acetonitrile:glacial acetic acid:triethylamine in one container. Retention time of acetaminophen is 3.058 minutes.

## Results

- The method was modified by combining solvents in one container to stop pulsing effects from viscosity differences in solvents shown in Figure 4.
- The retention time was too long, therefore a more polar mobile phase was made by adding more water, decreasing the retention time from 7.250 minutes to 4.175 minutes. Increased polarity decreased retention time necessary for kinetic studies.
- The final method included a 1.5 mL/min flow rate, and a mobile phase of 97.6:2:0.2:0.2 water:acetonitrile:glacial acetic acid:triethylamine.

## Results Continued

- A calibration curve of concentration and retention time was made with the 1, 2, 3, 4, 5 and 10 ppm solutions.

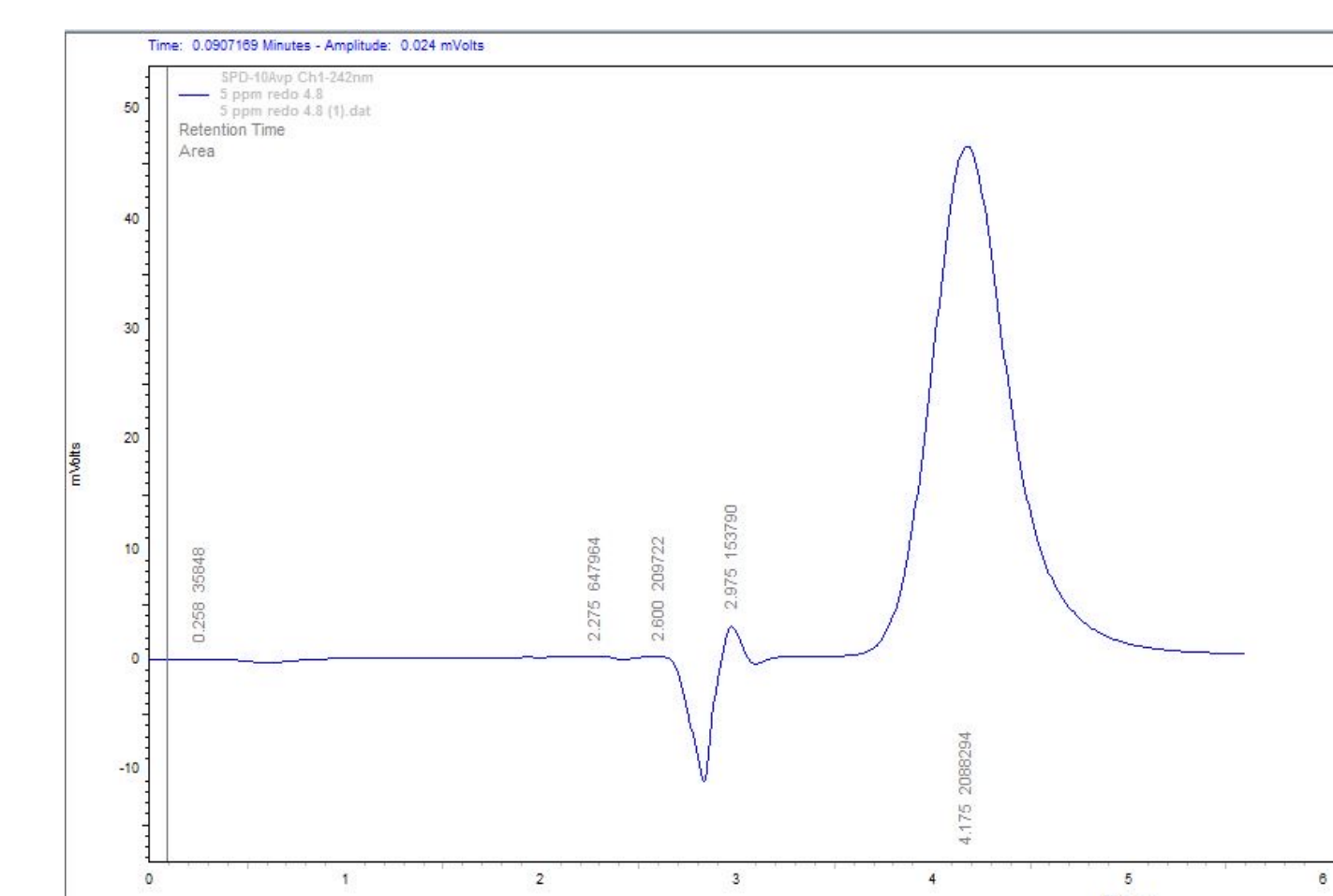


Figure 7: HPLC chromatogram of 5 ppm acetaminophen solution. 97.6:2:0.2:0.2 water:acetonitrile:glacial acetic acid:triethylamine in one container. Ideal chromatogram.

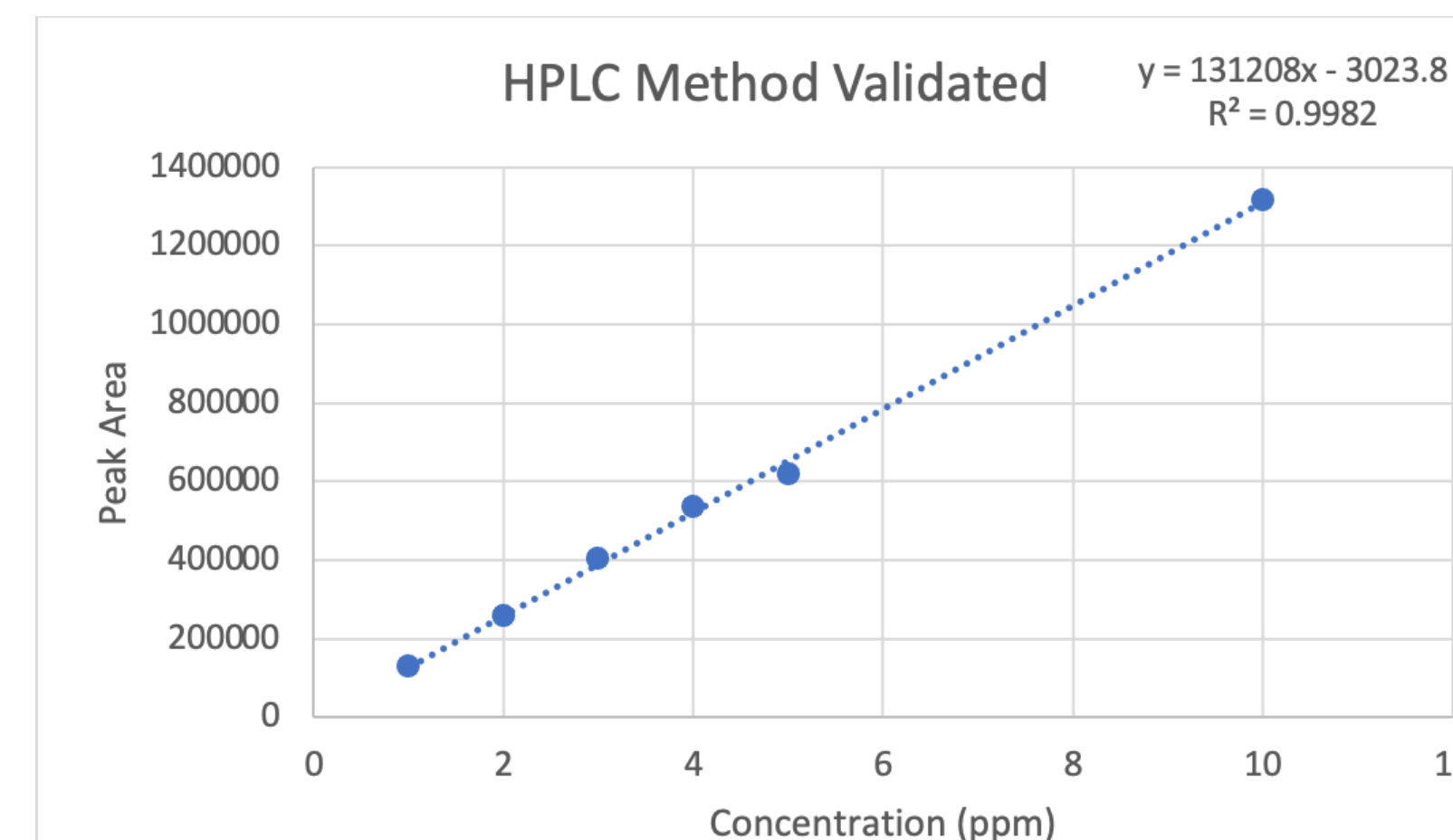


Figure 8: Calibration curve with a linear equation of  $y=131208x-3020.8$  and an  $R^2$  of 0.9982.

## Next Steps

- More research is needed in the kinetics of bleach degradation.
- A bleach tablet will be added to a known acetaminophen solution, and the concentration of acetaminophen will be monitored over time with validated HPLC method.



## References

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