



Comparison of Methanol, Water, and HPLC Mobile Phase as the Solvent for Quantification of Nicotine Using HPLC Method

Josie Traver, Dr. Emmalou Schmittzehe
Department of Biology and Chemistry
Morehead State University

Abstract

Nicotine is becoming increasingly popular in a variety of different forms. Commonly, cigarettes are used widely among many different age groups around the world for decades. In the present day, E-cigarettes are beginning to gain popularity, which is another delivery system of nicotine. The objective of this research experiment is to understand how varying concentrations of nicotine with quinoline mixed with three different solvents result in effectiveness. By using three different solvents, those being water, methanol, and HPLC Mobile in this experiment, we can determine how effective each one is based on the linearity of the calibration curve plotted using HPLC Method.

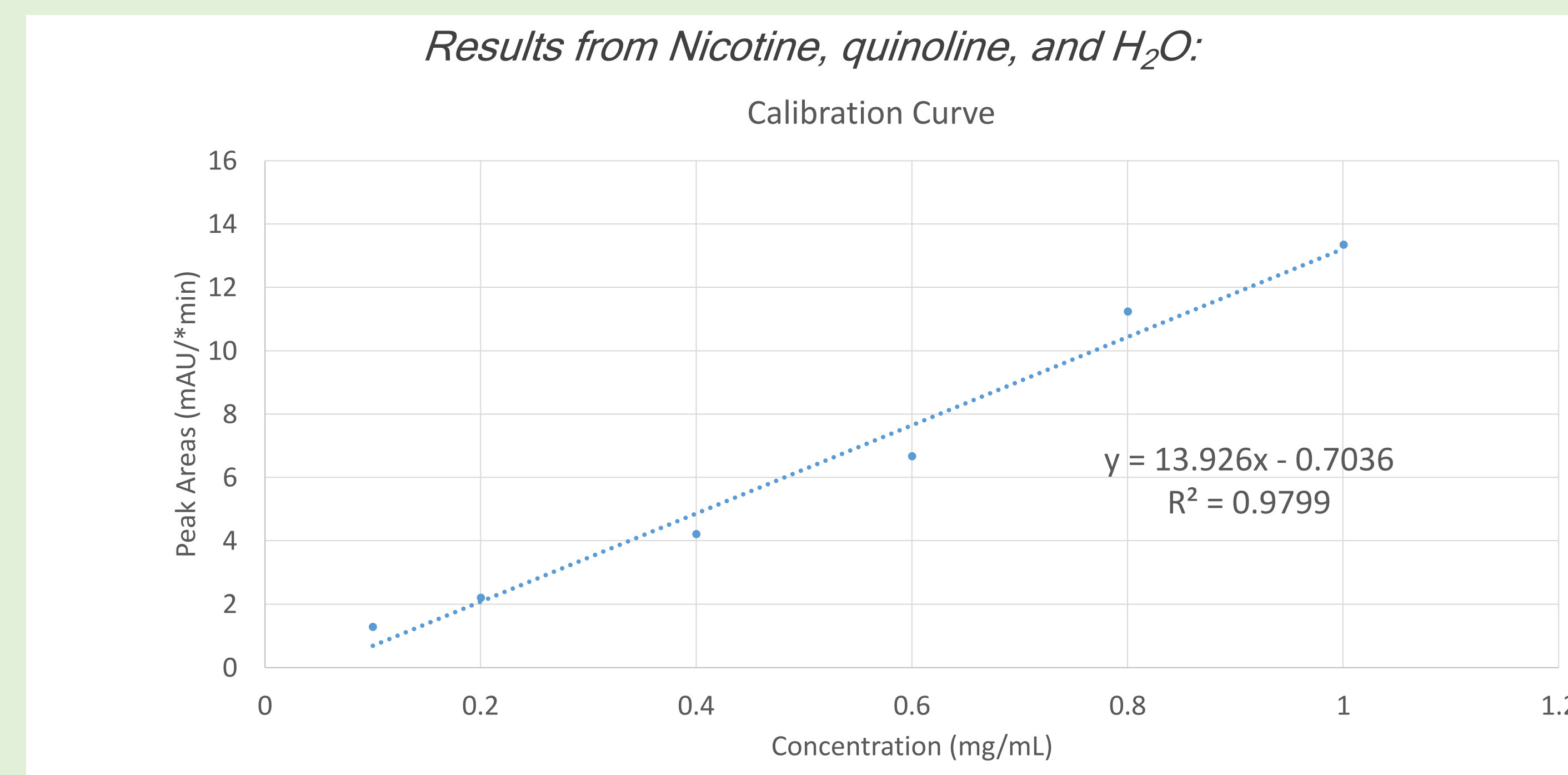
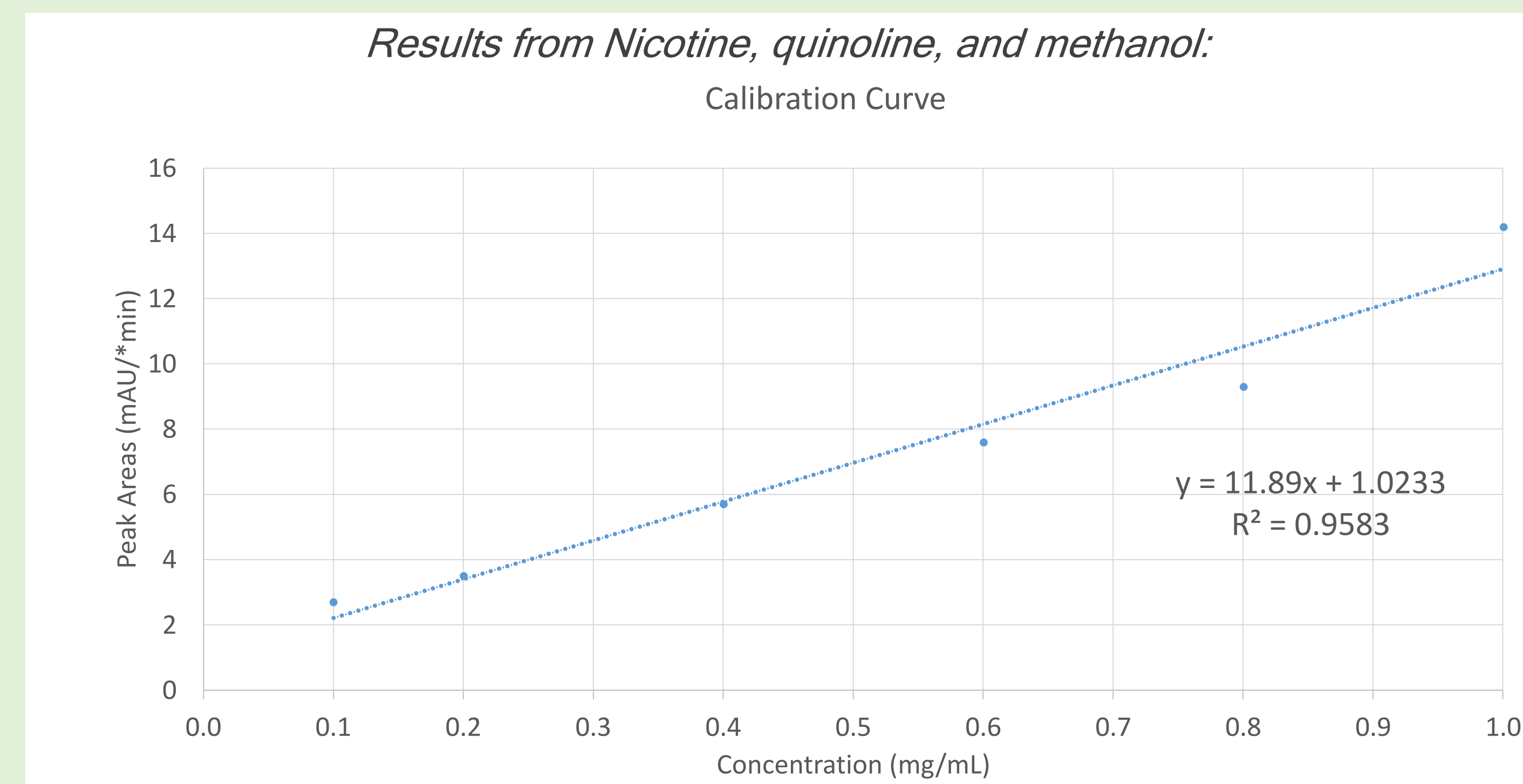
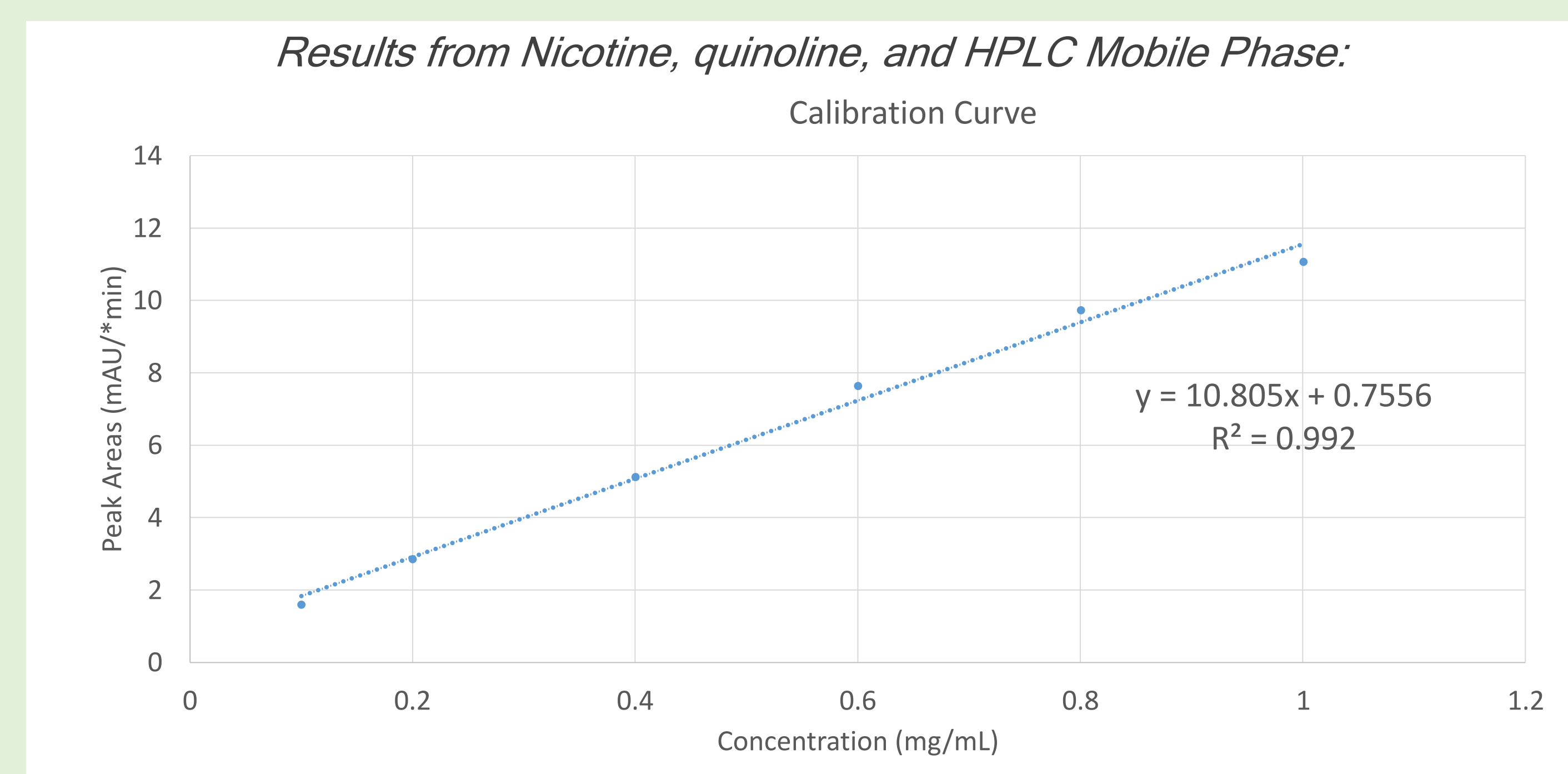
Introduction

Nicotine is a highly addictive chemical present in the tobacco plant. E-cigarettes, predominantly used among the adolescent population, are becoming increasingly popular. We can quantify the nicotine levels in unknown solutions using HPLC (high-performance liquid chromatography) by first preparing a calibration curve from standard nicotine samples. This experiment aims to understand the difference between using various solvents for the nicotine samples and how using one solvent may be more effective.

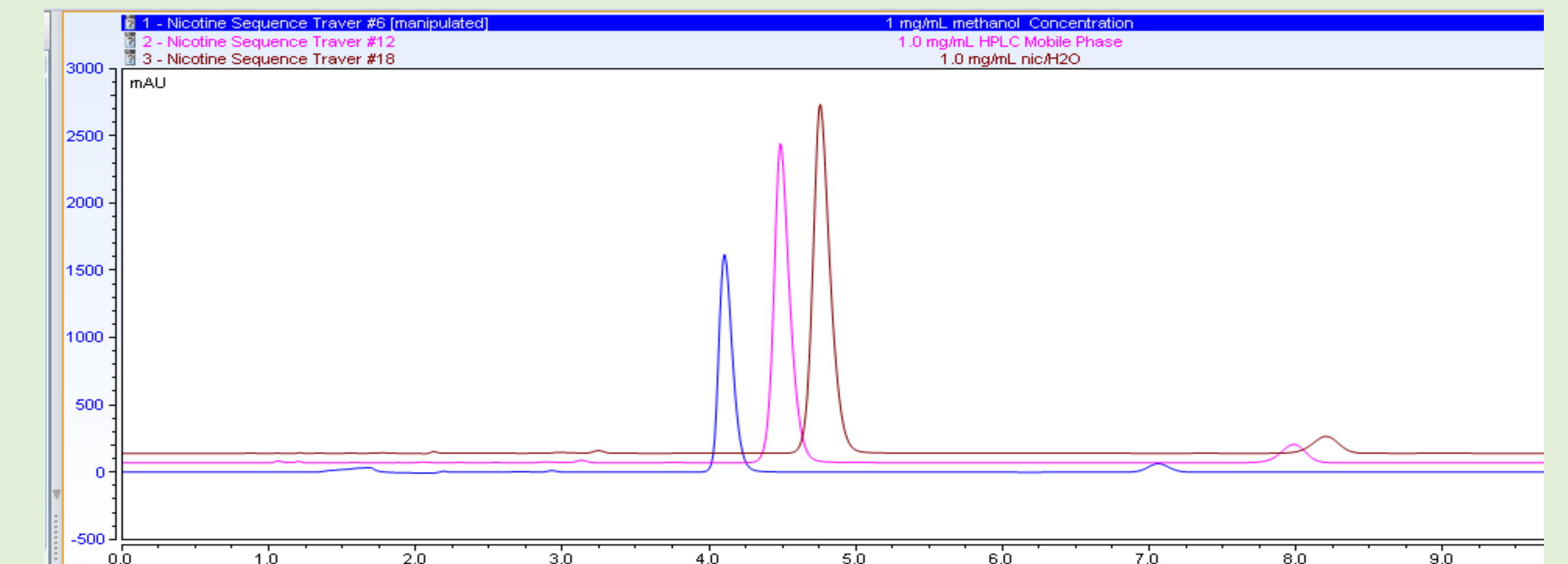
Materials and Methods

- Standard nicotine solutions were prepared in varying concentrations of nicotine mixed with three different solvents: methanol, water, and the HPLC mobile phase (a mixture of water, acetonitrile, and triethylamine).
- The nicotine standards were prepared from 1 mg/mL stock solutions in each solvent and were stored at 4°C. These included: 100, 200, 400, 600, 800, and 1000 µg/mL standards.
- 15 µg of the internal standard, 15 mg/mL quinoline, was added to all nicotine standards.
- Data were collected on a Thermo Scientific Vanquish HPLC equipped with a C₁₈ column and a diode array detector set at a UV wavelength of 254 nm.
- 10 µL aliquots of each standard were injected.
- The flow rate of the mobile phase was set at 1 mL/min and the run time for each injection was set to 12 minutes.
- The signal areas were used to create a calibration curve for nicotine with each solvent.

Results



- The HPLC chromatograms from the 1 mg/mL nicotine standards with each solvent are shown below:



Discussion

- When the R^2 is equal to 1.000, this means the line is completely linear. By comparing the R^2 values from each calibration curve, we can see that the HPLC Mobile Phase is closer to 1.000 while using the solvent methanol and water is further away from the cap R squared value of 1.000. Thus, it can be concluded that the use of the HPLC Mobile Phase created a more linear trend in comparison to the use of methanol and H₂O.
- Comparison of the HPLC chromatograms shows that the nicotine and quinoline come out at different times depending on the solvent, both shifting together and coming out either sooner or later depending on the solvent. In addition, the separation between the nicotine and quinoline peaks is slightly smaller when using methanol then when using water or the HPLC Mobile phase as the solvent.

References

Alhusban, A. A., & Ata, S. A. (2021). Simple HPLC method for rapid quantification of nicotine content in e-cigarettes liquids, *Acta Chromatographica AChrom*, 33(3), 302-307. Retrieved Apr 20, 2022, from <https://akjournals.com/view/journals/1326/33/3/article-p302.xml>