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Using HPLC and SPE to Determine the Effectiveness of Wastewater Treatment Plants in the Removal of Caffeine and its Metabolites

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Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project:
Using HPLC and SPE to Determine the Effectiveness of Wastewater Treatment Plants in the Removal of Caffeine and its Metabolites

Student Name: Erin Hair

Faculty Sponsor: Dr. Jeanne Franz

Department: Chemistry

Abstract
There is no natural source for caffeine even though a small amount is found in groundwater. This research is designed to determine the percentage of caffeine in groundwater and try to decide which wastewater treatment process, if any, is most effective at eliminating the caffeine. In order to determine this, a method that can measure caffeine at low levels was developed. UV-visible spectrophotometry (UV-vis) was used to find the lambda max of each sample at a 10-7 g/mL dilution in order to develop a High Performance Liquid Chromatography (HPLC) method. The caffeine standards were then run using HPLC with UV-vis detection. This produced a calibration curve in which peak area was plotted against concentration. Samples will be obtained from wastewater treatment plants, run using this method and then evaluated by comparing to the standards. A solid phase extraction method will be developed and used to further purify the samples.

The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 181, npeterson@winona.edu).

Student Signature: Erin Hair Date: 5/9/13

Faculty Sponsor Signature: Jeanne Franz Date: 5/9/13

Using HPLC and SPE to Determine the Effectiveness of Wastewater Treatment Plants in the Removal of Caffeine and its Metabolites

Erin Hain
CHEM 461
Spring 2013

Abstract

There is no natural source for caffeine even though a small amount is found in groundwater. This research is designed to determine the percentage of caffeine in groundwater and try to decide which wastewater treatment process, if any, is most effective at eliminating the caffeine. In order to determine this, a method that can measure caffeine at low levels was developed. UV-visible spectrophotometry (UV-vis) was used to find the lambda max of each sample at a 10^{-7} g/mL dilution in order to develop a High Performance Liquid Chromatography (HPLC) method. The caffeine standards were then run using HPLC with UV-vis detection. This produced a calibration curve in which peak area was plotted against concentration. Samples will be obtained from wastewater treatment plants, run using this method and then evaluated by comparing to the standards. A solid phase extraction method will be developed and used to further purify the samples.

Introduction

Caffeine is a manmade chemical, which means it is not found in nature. In reality, a household can produce thousands of milligrams of caffeine daily just by making a pot of coffee. Its presence can potentially have a negative effect on the environment.[1] Caffeine is found in many substances including coffee, soft drinks, tea, and pain pills. Caffeine is dispersed into the environment by dumping the liquids down the drain, or through urination. Caffeine is unmetabolized and possibly harmful to the environment.[2] It has the potential to increase the caffeine levels in water, which could have an effect on the aquatic wildlife, and possibly the humans, who consume the water. The process of determining caffeine levels in ground water is done using High Performance Liquid Chromatography (HPLC) and Solid Phase Extraction (SPE).[1] HPLC is the most widely used for the separation of substances[3] and SPE is used for the concentration of substances and the removal of interfaces from the substances of interest. According to previous research, first the HPLC method is used with no prior extraction methods[2] and then SPE is used as an extraction method along with the HPLC. The compounds being tested are Caffeine (CAS 58-08-2), Theobromine (CAS 83-67-0) and Theophylline (CAS 58-55-9).[1] Theobromine and Theophylline are known caffeine derivatives.[1] Overall, the findings from the different studies alluded to the fact that the levels of caffeine detected was in the part per billion range (ug/L).[2,4,5]

Caffeine in groundwater poses a potential threat to the environment and research needs to be done to determine its possible consequences. According to the National Exposure Research Laboratory, the magnitude of caffeine in wastewater, and the ramifications of it being there, are largely unknown.[2] A discovery that numerous drugs and personal care products from a wide spectrum of therapeutic and consumer-use classes, many having potent biochemical activity, are inadvertently released to the environment. These products are released in both direct and indirect ways. The direct ways include disposal from external application and the indirect ways consist of excretion, washing, and swimming. These indirect

ways are primarily due to treated and untreated sewage effluent, and also by terrestrial runoff (confined animal feeding operations). The main source for the drug quantity, including caffeine, in wastewater is due to municipal or domestic sewage, opposed to hospital sewage. The extent of the toxicological effects of these substances in wastewater and groundwater are not very well known. There are no requirements for the monitoring of these products in wastewater in the United States. This could be because there is a high occurrence of untreated sewage discharge, which would allow a potential for higher concentrations in a greater variety of chemicals.[4]

It is important to understand how a wastewater treatment plant works in order to determine their efficiency. There is primary, secondary, and tertiary treatment involved in each treatment. Each treatment has substations. These include screening, pumping, aeration, clarification, and disinfection. This can be seen in Figure 1 below.

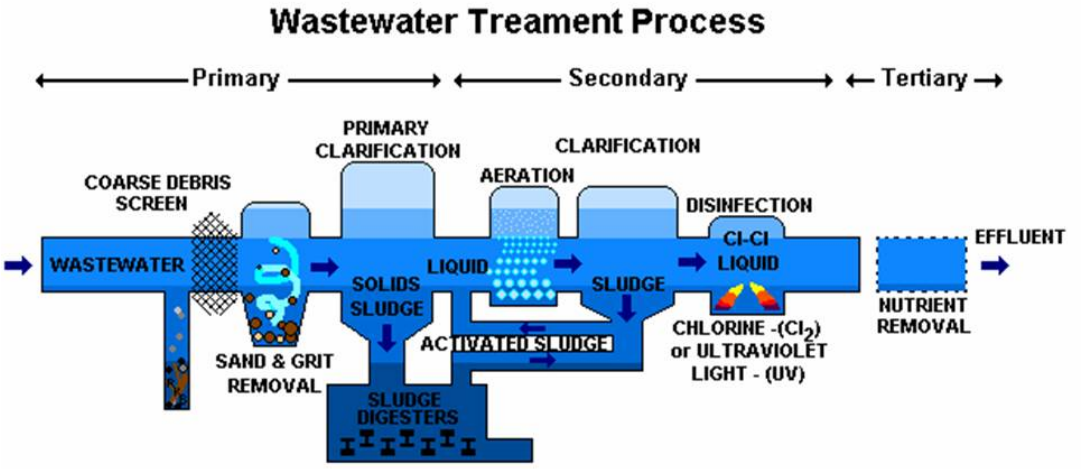

Figure 1: Wastewater Treatment Process[6]

In Beijing, China a study was done that included the testing of caffeine concentration in four wastewater treatment plants. Solid phase extraction was used to take samples from the wastewater and analyze them. Like other studies done before this, liquid chromatography and mass spectroscopy was used to analyze these samples. The results of this test showed that primary treatment removed almost no caffeine from the sample. Secondary and tertiary treatment proved to be more successful in lowering the caffeine concentration of the samples. The results also showed that ozonation and reverse osmosis were very effective in removing caffeine and also showed that these processes may be the main contributor in removing pollutants from wastewater.[5]

There are many steps in wastewater treatment, and many different methods can be used to clean water. Research has been done on the effectiveness of wastewater treatment plants. There is no regulation on caffeine concentration in wastewater. This means that there is no process in the wastewater treatment plants that specifically removes caffeine. The proposal is to see if any processes incidentally remove caffeine. This will then be used to find the most efficient

combination of wastewater treatment processes (primary, secondary, tertiary). This will be done using HPLC and SPE.

Materials and Methods

In order to interpret the results obtained in this experiment, standards need to be used as a baseline. The standards used in this experiment were caffeine, theobromine, and theophylline. These structures can be seen in Figures 2-4.[7]

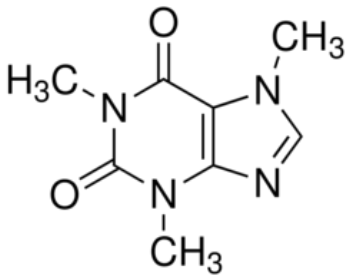

Figure 2: Caffeine

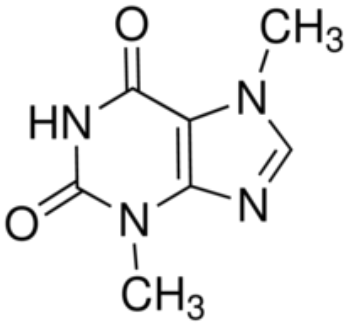

Figure 3: Theobromine

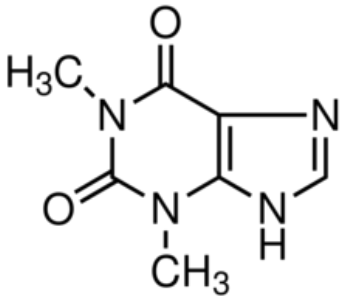

Figure 4: Theophylline

These standards were diluted to 10^{-7} fold and tested using the Shimadzu UV-vis spectrophotometer in order to determine the lambda max for each caffeine derivative. This information was used to determine the HPLC machine settings. The HPLC was set using an isocratic method with an acetonitrile solvent. This solvent was made up of a 10% (100mL acetonitrile diluted to 1L) to 90% (5mL of acetic acid in 1L HPLC grade water) ratio. The timetable for the HPLC operation can be seen in Table 1. This procedure was run for ten minutes through a C18 chromatographic column.

Time	Module	Action	Value
0.1	Pumps	Pump B Conc.	25.0
9.99	Pumps	Pump B Conc.	25.0
10.0	Controller	Stop	

Table 1: Isocratic Time Program

This isocratic mobile phase was not producing useable results, so a binary mobile phase was implemented. The binary method was made up of 25% (acetonitrile) and 75% (mixture of 5 mL acetic acid in 1L of water) each in a different pump, coming together to create the mobile phase. The timetable can be seen in Table 2. The standards were tested using this method and calibration curves were made from the data.

Time	Module	Action	Value
0.1	Pumps	Pump B Conc.	25.0
9.99	Pumps	Pump B Conc.	25.0
10.0	Controller	Stop	

Table 2: Binary Time Program

Once the standards have been run and produce credible results, the unknown solutions will be obtained and tested. Then a SPE method will be developed to further purify the unknown samples.

Results and Discussion

Four different dilutions of each of the three standards was made. The first dilutions were 10^{-3} g/mL and the UV-vis data from these solutions was unusable. Due to this, the solutions were diluted to 10^{-5} g/mL. This again produced results that were slightly high and the standard curves were not as linear as they should have been. These absorbance values for each sample can be see in Table 3 and the calculation for the dilution can be seen in Sample calculation #1.

Caffeine	Concentration	Absorbance	Lambda Max
c1	2.00E-08	0.201	204
c2	4.00E-08	0.222	
c3	6.00E-08	0.267	
c4	8.00E-08	0.254	
Theobromine	Concentration	Absorbance	
b1	2.00E-07	0.839	203
b2	4.00E-07	1.251	
b3	6.00E-07	1.459	
b4	8.00E-07	1.767	
Theophylline	Concentration	Absorbance	
p1	2.00E-07	0.504	202
p2	4.00E-07	1.047	
p3	6.00E-07	1.37	
p4	8.00E-07	1.667	

Table 3: UV-vis Data

Sample Calculation #1: Dilution of samples: 10^{-3} to 10^{-5} g/mL
$M_1V_1=M_2V_2$
$(2x10^{-3}\ g/mL)(V_1)=(2x10^{-5}\ g/mL)(50\ mL)$

*V_1=0.5 mL *this is too little of volume to transfer accurately, so the dilution was done in two steps*

Step 1:
$M_1V_1=M_2V_2$
$(2x10^{-3}$ g/mL)(V_1)=($2x10^{-4}$ g/mL)(50 mL)
V_1=10 mL
Step 2:
$M_1V_1=M_2V_2$
$(2x10^{-4}$ g/mL)(V_1)=($2x10^{-5}$ g/mL)(50 mL)
V_1=5 mL

Since the results from this run were not as good as what was wanted from a standard curve, the samples were again re-made and diluted to 10^{-7} g/mL. This dilution produced desirable lambda max, absorbance, and standard curve values. These can be seen in Table 4 and Figures 5-7.

Caffeine	Concentration (g/mL)	Absorbance	Lambda Max
c1	2.00E-07	0.175	245, 273
c2	4.00E-07	0.218	
c3	6.00E-07	0.276	
c4	8.00E-07	0.307	
Theobromine	Concentration (g/mL)	Absorbance	
b1	2.00E-07	0.162	244, 272
b2	4.00E-07	0.294	
b3	6.00E-07	0.357	
b4	8.00E-07	0.397	
Theophylline	Concentration (g/mL)	Absorbance	
p1	2.00E-07	0.123	244, 271
p2	4.00E-07	0.18	
p3	6.00E-07	0.271	
p4	8.00E-07	0.275	

Table 4

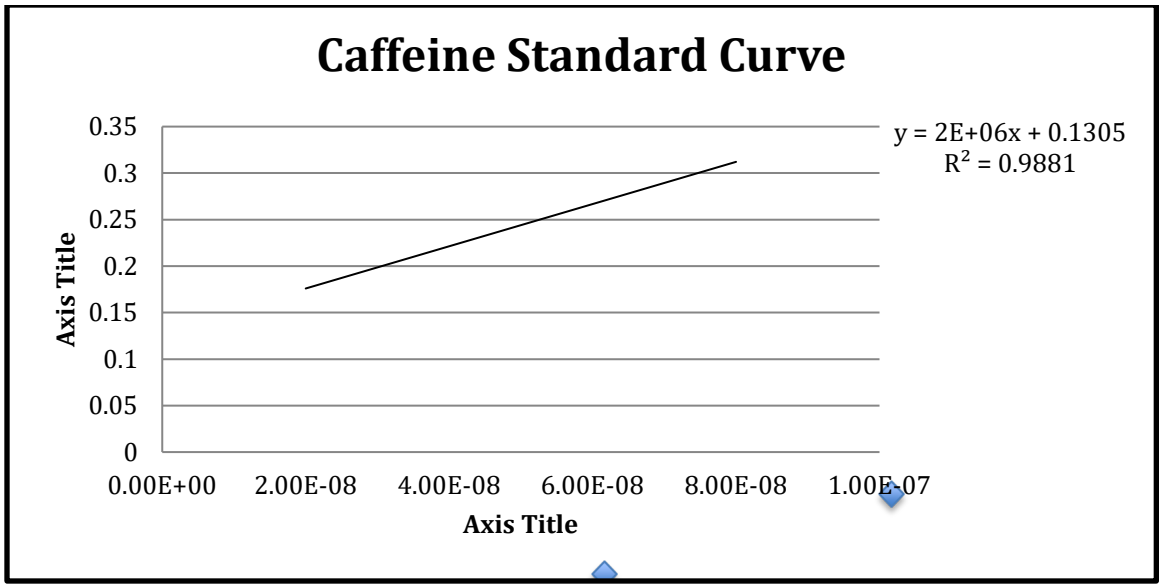

Figure 5

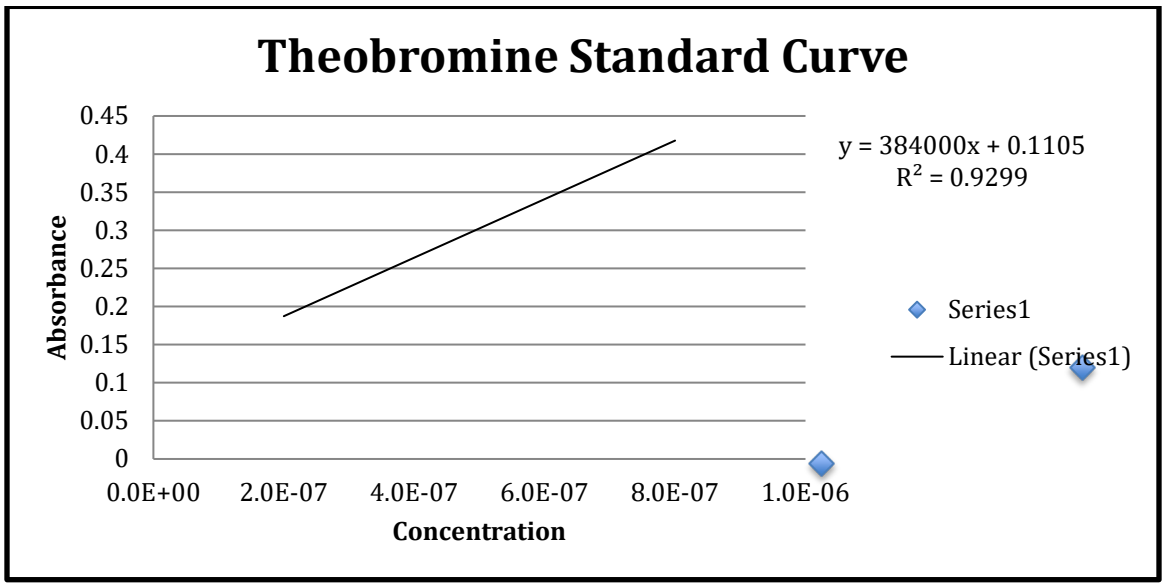

Figure 6

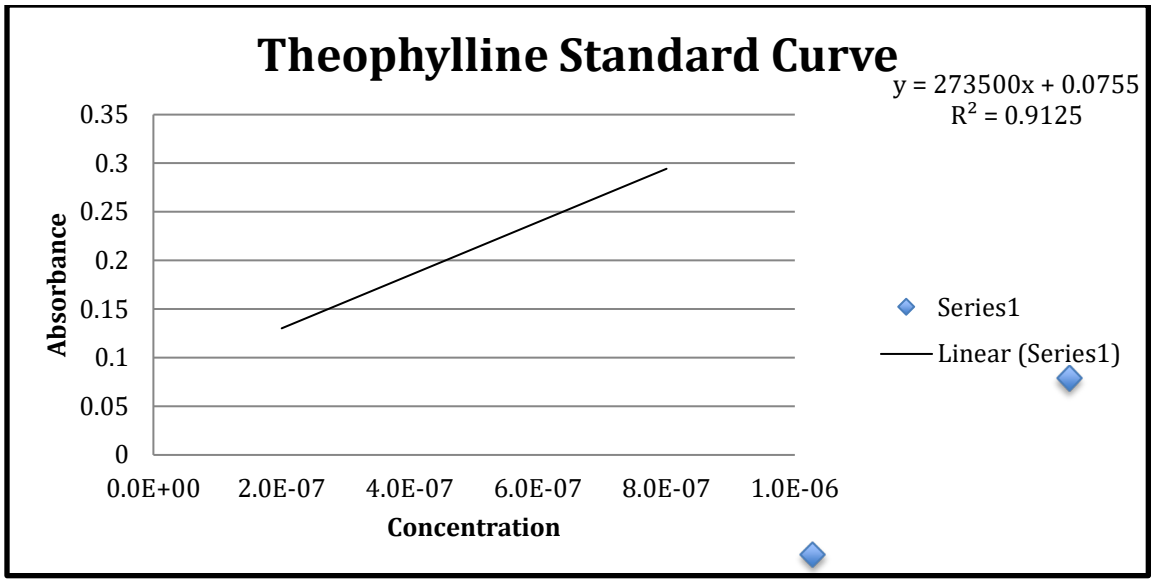

Figure 7

The data from the samples that were run on the UV-vis spectrophotometer was used to make the template for the HPLC. The first few weeks, the HPLC was malfunctioning and produced only a flat line when the caffeine standard 4 was run. The next week, the sample stopped at 3 minutes consistently for multiple runs. Due to this, a new program was made and this solved the problem. The new program used a binary method instead of an isocratic, but both pumps were in the same solvent so it was just like an isocratic method. With this new method, the 10^{-7} g/mL caffeine solution was run successfully and it was concluded that the solutions were too dilute and needed to be remade. The solutions were then remade to 10^{-6} g/mL and the same conclusion was made. The solutions were remade to 10^{-5} g/mL and a good peak formed around 6.5 min. This peak, however, was still too dilute so the solutions were made again to a concentration of 10^{-4} g/mL. These solutions were run using the HPLC and did not produce any useable results. A new column and guard column was placed into the HPLC in hopes that this would help produce useable results. It was also determined that the current mobile phase was not polar enough to extract the standards from the stationary phase. A new mobile phase was developed which included Pump A being set at 25% and containing acetonitrile and Pump B being set at 75% and containing a mixture of acetic acid in water. This mobile phase was successful and produced credible peaks. These can be seen in the Appendix along with a representative caffeine chromatograph in Figure 8. From these chromatographs, the peak area was used to make a calibration curve. Theobromine produced a good calibration curve, but theophylline and caffeine did not. The Theobromine calibration curve can be seen in Figure 9. Future research needs to be done to investigate the cause of this. Glassware contamination could be a possible option.

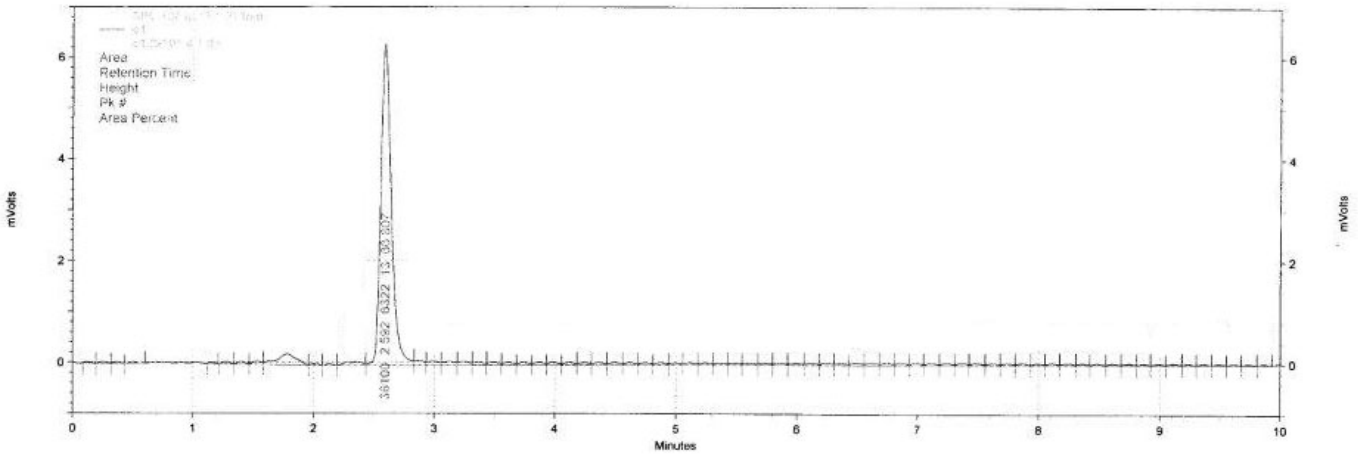

Figure 8: Representative Caffeine Chromatograph

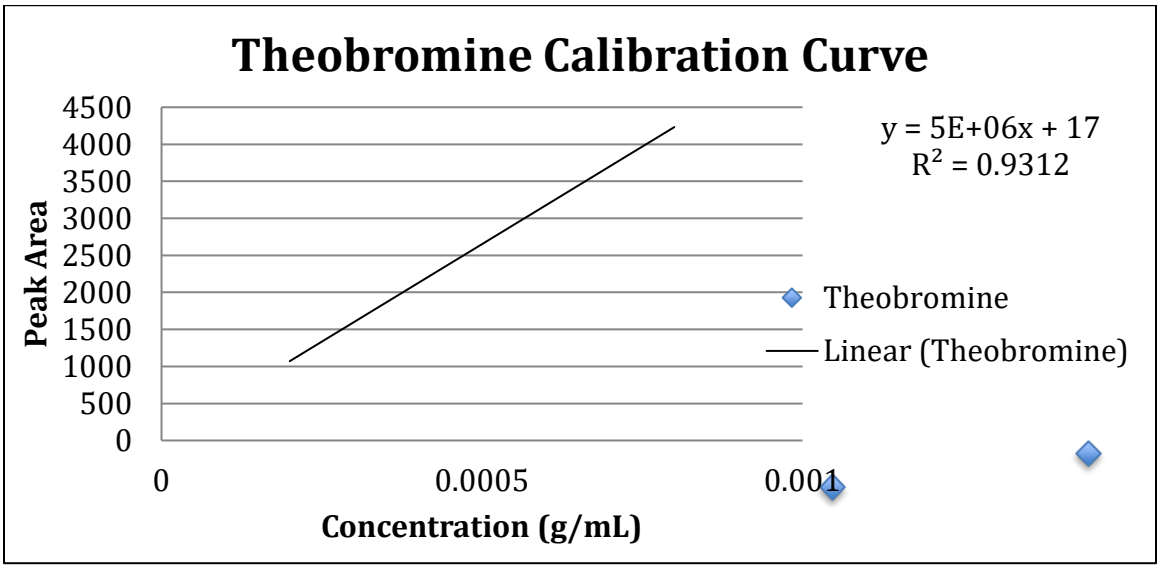

Figure 9

Conclusion

Not much is known about caffeine in groundwater. This research was designed to determine the amount of caffeine in groundwater and which wastewater treatment process, if any, is most effective at eliminating the caffeine. UV-vis spectrophotometry was used to find the lambda max of each sample in order to develop the HPLC method. The lambda max for the samples was determined to be 273 nm (determined from samples of 10^{-7} g/mL). An HPLC method was developed and set to run for ten minutes using a binary mobile phase. A Theobromine calibration curve was created using the chromatographs obtained from the HPLC. This equation was found to be y = 5E+06x + 17. Future research will include the

development of a solid phase extraction method in order to purify and concentrate samples.

References

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Detailed Budget for Account # 213-779 Erin Hain

C-18 column for HPLC (replacement of old, worn out one)
$521.05

Student research posters for Research celebration (for three students in group)
$ 72.00

Flow cell for HPLC (to replace old, stained one) $ 6.95
note: flow cell cost was split with other accounts

Total expenditures: $600.00