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CAFFEINE: AN ENVIRONMENTAL FORENSIC TRACER IN A HOUSEHOLD SEPTIC TANK AS AN INDICATION OF HUMAN ACTIVITY

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M.S. degree in Criminal Justice

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Major Professor's Signature

5/3/05
Date

CAFFEINE: AN ENVIRONMENTAL FORENSIC TRACER IN A HOUSEHOLD SEPTIC TANK AS AN INDICATION OF HUMAN ACTIVITY

By

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ABSTRACT

CAFFEINE: AN ENVIRONMENTAL FORENSIC TRACER IN A HOUSEHOLD SEPTIC TANK AS AN INDICATION OF HUMAN ACTIVITY

By

Tavy J. Wade

The suitability of caffeine as an anthropogenic marker as forensic evidence of human activity in an anaerobic environment was examined in this study. An anaerobic degradation rate for a slug input of caffeine into a household septic tank as well as aerobic and anaerobic degradation rates of caffeine added to the septic tank water in laboratory reactors were determined. The study was completed in Okemos, Michigan in an effort to understand the kinetics of caffeine in the aquatic environment. The analytical method used to determine the concentration of the caffeine included high-pressure liquid chromatography (HPLC) with a detection limit of 0.01-ppm. Triplicate samples were taken from the septic tank twice per day for 11 days and analyzed for caffeine and bromide while the caffeine concentrations of the anaerobic and aerobic reactors were monitored for one week. The anaerobic septic tank and aerobic reactor caffeine degradation rates of -0.0006mg/L-hr and 0.077mg/L-hr respectively, show that aerobic processes are likely to govern the degradation of caffeine by first-order kinetics. With a dispersion number of 0.03, a coefficient of axial dispersion of 2.19×10^{-2} ft^2/hr, and a caffeine degradation rate of 0.0064 mg/L-hr in the tank, it appears that dispersion plays a small role in the degradation of caffeine in a septic tank. This study provides evidence that when mixing and flushing are taken into consideration, degradation of caffeine under anaerobic conditions occurs slowly and with further testing caffeine may be validated as an anthropogenic marker of human activity in an anaerobic environment.

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INTRODUCTION

Forensic scientists and members of law enforcement are constantly searching for additional tools to assist them in their criminal investigations. Utilizing caffeine as an environmental tracer may provide a unique manner in which scientists can glean important information concerning a specific type of crime scene. This could include an instance in which a person or persons goes missing. Perhaps the missing person lives in a rural area or residence that employs a septic tank for wastewater removal and it appears that the residence has not been disturbed for some time. The detection of caffeine in the septic tank points towards recent human activity at the residence. This would be very helpful in providing investigators with a lead as to where to begin their search for the missing person or persons.

In another possible scenario, suppose a boat is found abandoned ashore with no sign of any persons in the immediate area. Did something happen to the person or person(s) elsewhere and the boat drifted here on its on own or has there been recent human activity in the area? Similarly, articles of clothing or items that would normally be used by human beings are found near a body of water. Did animals or weather carry these items here, or is it more likely that humans have been in the vicinity? If an individual has consumed coffee or soda and disposed of them in the area, the detection of caffeine would indicate that there indeed has been human activity nearby. However, little research has been done in the area of environmental tracers as an instrument to aide forensic investigators.

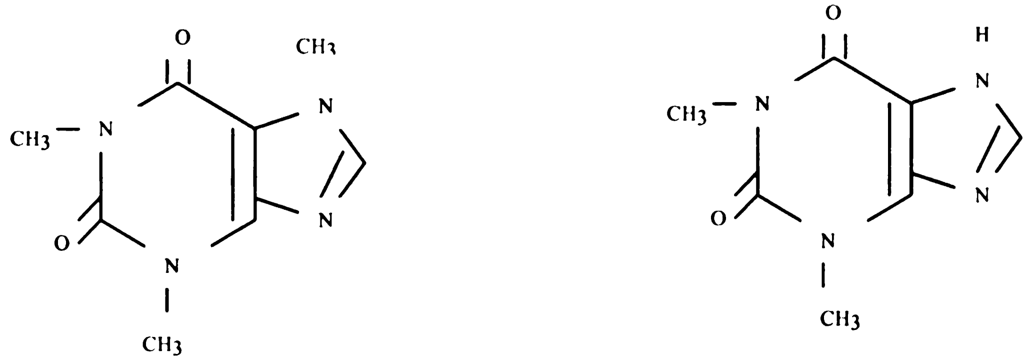
There have been many previous attempts to use environmental tracers as indicators of domestic wastewater contamination including fecal materials and chemical markers such as pharmaceutical and personal care products (PPCPs) and food. One PPCP that has been studied for use as an environmental tracer is caffeine, but there is little information about its fate in the environment and not yet enough evidence to show the usefulness of caffeine as a tracer (Gardinali and Zhao, 2002). However, in instances in which a rural area with a septic system as its sole source of wastewater removal is the scene of a crime, the detection of caffeine in the septic tank may confirm that indeed there has been recent human activity at the residence. This question sometimes arises when assessing certain aspects of a crime such as if people were present at the suspected crime scene, at or near the time of the crime. Clearly, there is a need to further investigate the mode in which caffeine degradation is most efficient in a septic tank before this suggestion can be of value to forensic analysis.

The kinetics of caffeine reactions have been studied in various wastewater and natural water regimes, however little attention has been paid to caffeine degradation in anaerobic environments such as in a septic tank (Seiler et al.1998). One objective of this study was to determine a degradation rate for caffeine in a septic tank. This was done in two phases: a laboratory study utilizing water from a septic tank and a field experiment with the addition of caffeine and bromide into the same septic tank. The laboratory study involved an anaerobic and aerobic experiment using septic tank water to determine laboratory degradation rates for the caffeine, which were compared to the field degradation rate. The bromide, which served as a conservative tracer, was used as an indicator of the amount of mixing occurring in the tank. This objective tested the major hypothesis that caffeine can be used as an indication of human activity in an anaerobic environment.

BACKGROUND

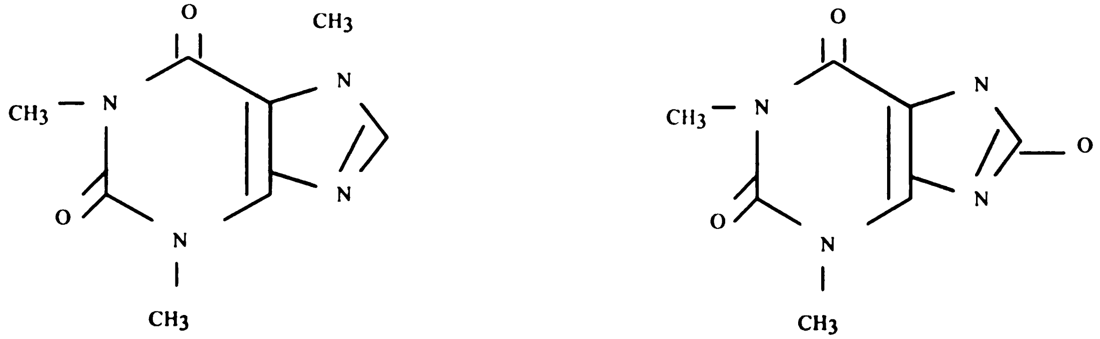
Caffeine is an alkaloid and is the major constituent of coffee, tea, cacao, and cola, which contain about 100, 50, 10, and 40 mg of caffeine per serving, respectively (Buerge et al. 2003). The global average consumption of caffeine is 70 mg per person per day varying largely among different countries. However, the major distribution of caffeine into the environment is from coffee. One pot of coffee generally contains about 1500 to 1800 mL and therefore has the potential to be discharged in large quantities daily to a household septic system, wastewater via urine, or directly. Caffeine is partially metabolized in humans by the hepatic cytochrome P450 enzyme. Theophylline (1,3-dimethylxanthine), paraxanthine (1,7-dimethylxanthine), 1,3,7-trimethyluric acid and other byproducts are produced by the oxidative N-demethylation and/or ring oxidation action caused by the enzyme, which are excreted along with undegraded caffeine in urine. See Figure 1 for an illustration of the chemical structures of caffeine and its degradation products.

Caffeine (1,3,7-trimethylxanthine) is ubiquitous in the human diet, which makes it an ideal candidate to trace in the environment. Whether it is by coffee, tea, caffeinated soft drinks, chocolate, and medications excreted in urine, or disposing of unconsumed caffeine directly, caffeine and its metabolites have the potential to be discharged into the environment in large amounts. However, caffeine in the aqueous environment is usually found in the ng to µg per liter range. This could be due to the small percentage of unmetabolized caffeine that enters the environment through human waste. Only 3% of caffeine is excreted unmetabolized in urine as stated in Seiler (1998) and 0.5-10% unmetabolized as stated in Buerge (2003). Despite this, caffeine by-products are seldom



Caffeine
(1,3,7-trimethylxanthine)

Theophylline
(1,3-diemethylxanthine)



Paraxanthine
(1,7-dimethylxanthine)

1,3,7-trimethyluric acid

Figure 1 Chemical structures of caffeine and caffeine by-products.

detected in wastewater. Their low detection in wastewater may be contributed to the idea that aerobic environments promote rapid degradation of caffeine and caffeine by-products are even less stable than caffeine. Does this also mean that caffeine and or caffeine by-products will be difficult to detect in a household septic tank?

Few studies have been completed to show the efficiency of caffeine elimination in WWTPs and demonstrate the degradation of caffeine in natural water systems and there are even fewer studies on the fate of caffeine in septic tanks. However, it is important to note one study reported in Seiler (1998) in which well water was sampled from areas that utilized septic systems for wastewater management needs. The assumption before completing this study was that because of the manner in which it persisted in the body, caffeine acted conservatively (did not decay) in wastewater and could therefore be used as an accurate tracer of recharge from domestic wastewater. Two analysis techniques were used to analyze the coffee including HPLC with no extraction and gas chromatography mass spectrometry (GCMS) using 1-L samples extracted with methylene chloride by continuous liquid-liquid extraction. The study concluded that caffeine is not conservative and the complete degradation of caffeine most likely took place in the septic tank, which is anaerobic, or in the aerobic leach field. Ultimately, "the low concentrations of caffeine and pharmaceuticals found in wells with high nitrate concentrations during this study indicate that processes of catabolism, dilution with native groundwater, and sorption to soils substantially reduce the amount of these chemicals in household wastewater that make it through a septic system and unsaturated zone to the water table," (Seiler et al. 1998).

It is important to have an understanding of the manner in which anaerobic and aerobic degradation of caffeine occurs, however the kinetics of caffeine in the environment, specifically in septic tanks, is not completely known. Aerobic degradation seems to be the main factor influencing the metabolism of caffeine in an aqueous environment since it has been shown that in the absence of oxygen-demanding enzymes reacting through a biphasic pathway, the breakdown of caffeine in wastewater is not likely. In a study done by Ogunseitan in 1996, caffeine was added to raw wastewater, primary effluent, secondary effluent, and creek water samples and incubated for three days and the concentration of caffeine-degrading bacteria increased. This increase in bacteria occurred at the same time that caffeine concentrations began to decrease although creek water samples did not degrade at all. Caffeine concentrations were confirmed by spectrophotometric scan at an absorbance of 273.5 nm, which was used to determine a rate constant of 0.0029 mg/mL-day. Ogunseitan suggested, "Rate-limiting biodegradation kinetics within activated sludge reactions is one of the major reasons why caffeine is routinely found in treated sewage effluents." It was expected that caffeine degradation would be very slow when these concepts were applied to caffeine degradation under anaerobic conditions such as in a septic tank. However, other factors such as mixing, dilution, and flushing are all likely to contribute to the decrease in caffeine concentrations in a septic tank. Mixing refers to how well the caffeine mixes with the other chemicals in the wastewater. Dilution of the system is a result of continuously adding wastewater from the household, which in turn dilutes the amount of caffeine in the tank, relative to the other chemical wastes in the system. Flushing of the septic tank entails removal of caffeine from the system due to flushing of toilets, showering, and usage of the sinks from members of the household. All of these processes lead to an increase in the degradation rate or breakdown in caffeine. An increase in the rate that caffeine breaks down will lead to a decrease in the concentration of caffeine in the septic tank.

METHODS AND MATERIALS

Preliminary Study

A feasibility study was first carried out during the months of May through June 2004 to determine if caffeine could be identified downstream of the East Lansing Wastewater Treatment Plant (ELWWTP). As stated previously, caffeine detected in surface water downstream of a wastewater treatment plant may indicate wastewater contamination. The outfall from the treatment plant, which empties into the Red Cedar River, was measured on June 2, 2004 and contained a caffeine concentration of 0.09-ppm, but caffeine could not be detected from the surrounding areas downstream of the river. The raw sewage was then tested and caffeine was measured at a concentration of 0.122-ppm. It was thought that since the raw sewage contained caffeine that maybe there would be measurable amounts of caffeine upstream from the treatment plant.

On June 8, eleven samples were taken along a two-mile stretch of the river on the campus of Michigan State University upstream from the ELWWTP and analyzed. A caffeine concentration of 0.1-ppm was detected in only one location in the portion of the river behind the Kellogg Hotel and Conference Center. It was thought that caffeine was discovered here because of the numerous conferences and meetings that take place in Kellogg on a daily basis and is probably where the largest concentration of caffeine is discharged from the campus. Closer sampling points (an average of 40-200 feet apart) within the portion of the river behind the Kellogg Center were then sampled on June 23 in an attempt to determine a caffeine degradation rate, however no additional caffeine was detected.

Last, wastewater effluent from an area 100 miles away in Macomb County was also examined for caffeine. Upstream and downstream samples as well as the outfall from the Mount Clemens, MI Wastewater Treatment Plant were sampled and tested for caffeine to determine if there was possible wastewater contamination into the Clinton River where the treatment plant empties. The upstream and downstream samples were taken approximately 200 meters from the outfall location, but no caffeine was detected from any of these sampling sites.

Caffeine concentrations beyond their entry point into the rivers could not be profiled, which demonstrate the likelihood that caffeine concentrations do not persist long in aerobic conditions. Nevertheless, any caffeine that was present in the test areas downstream of the wastewater treatment plant outfalls, were below the detection limit of the HPLC used in this investigation. Therefore, an anaerobic study of caffeine in a household septic tank was conducted as well as an aerobic and anaerobic laboratory reactor study with the aims of establishing a caffeine degradation rate constant, comparing anaerobic and aerobic degradation rates, and establishing which process governs the breakdown of caffeine in the environment to confirm if caffeine could be used as an accurate implication of human activity in an anaerobic environment.

Septic Tank Study

The analytical method used to detect caffeine for this experiment was adapted from Piocos and de la Cruz, 2000. It involved using an HPLC scheme with a Gilson model 303 HPLC set up with a Supelco Discovery C_8 column (25 cm x 4.6 mm, 5μm) using a mobile phase of 10% acetonitrile (ACN) and 90% distilled deionized (DDI) water with a flow rate of 1mL/min. The UV detector was set at a wavelength of 274 nm, which

Piocos and de la Cruz have reported as the absorption maximum of caffeine. A caffeine calibration curve was established after making a 100-ppm stock solution of caffeine using caffeine anhydrous std-$C_8H_{10}N_4O_2$: $\geq$99% HPLC in 200-mL DDI water. The appropriate serial dilutions were then made to make six more caffeine standards, which included 0.01-ppm, 0.05-ppm, 0.1-ppm, 0.5-ppm, 1-ppm, and 2-ppm solutions all in 25-mL final volumes of DDI water. See Appendix A for caffeine calibration chromatograms. This range was chosen to encompass the range of the background caffeine concentration in the septic tank (0.08-ppm) and the 1-ppm concentration of caffeine that was added to the septic tank. A 20-µL injection of each standard was then made into the HPLC using an Alcott autosampler with corresponding glass vials and polypropylene caps. All glassware used in this study was washed with soap and water and triple rinsed with ultrapure DDI water.

Similarly, a calibration curve was created for bromide in which sodium bromide (NaBr) was used. Sodium bromide was used to monitor the amount of mixing occurring in the tank. Mixing can be measured by a dispersion rate, which will be discussed in the results section. Caffeine could not provide an effective manner in which to measure dispersion because it decays in the environment as shown in the literature. Sodium bromide was chosen as a conservative tracer, or tracer that does not decay, and it is easy to measure. The NaBr was added to the septic tank in combination with the caffeine and also followed the same sampling and measurement schedule as caffeine. The method used to construct the bromide calibration curve was partially developed from the standard method, Determination of Anions by Ion Chromatography, using a Dionex Series 2000i/SP Ion Chromatograph (IC) with a Dionex IonPac AS4A-SC (4 x 250 mm) analytical column, bicarbonate eluant, and a 50-µL injection. An Alcott autosampler was also used with the same vials as described above. A 100-ppm stock solution of NaBr was made in 200 mL of DDI water. The bromide calibration curve consisted of 1-ppm, 2-ppm, 5-ppm, and 10-ppm standards and were serially diluted using final volumes of 25-mL. Very small concentrations of bromide do not show very defined chromatogram peaks on the IC so a bromide concentration of 5-ppm was added to the septic tank as a slug input. A mass of 56.8g of NaBr was added to 2-L of DDI water to achieve the 5-ppm concentration, which was based on the 1500-gal volume of the septic tank:

$$1500 \text{ gal} * \frac{3.785 \text{ L}}{1 \text{ gal}} * \frac{5\text{mg}}{\text{L}} * \frac{1\text{g}}{1000 \text{ mg}} = 28.38 \text{ g in 1-L of DDI}$$

$$28.4 \text{ g} * 2 = 56.8 \text{ g of NaBr in 2-L of DDI}$$

The septic tank utilized for this study was located at a residence in Okemos, MI. The dimensions of the tank were 13. 4 ft x 3ft x 5 ft with a 1500-gallon capacity. A piece of High-Density Polyethylene (HDPE) tubing was inserted into the effluent pipe that was long enough to reach above the ground surface and a peristaltic pump with rubber tubing was connected to the HDPE tubing to obtain septic tank samples. See the schematic of the sampling setup depicted in Figure 2.

It was decided that sodium bisulfate ($NaHSO_4$) would be added to septic tank samples to ensure that only degradation of caffeine within the tank would be measured. Granular $NaHSO_4$ was used to prevent further growth of microorganisms in the septic tank by lowering the pH of the wastewater. It was chosen because it is less toxic to work with than other chemicals such as formaldehyde, which would also serve this same purpose.

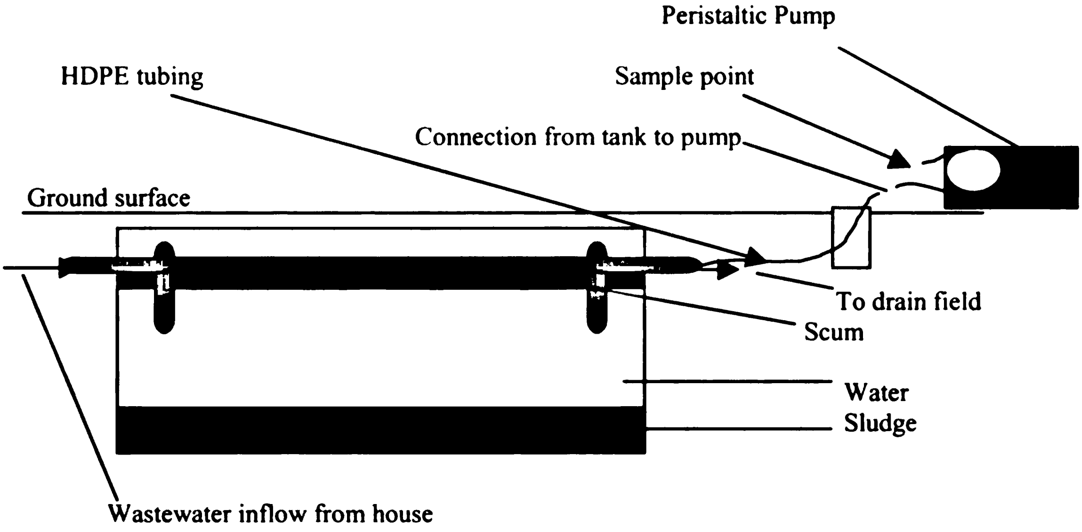


Figure 2 Sampling setup of residential septic tank in Okemos, MI[1].

A 500-mL septic tank sample was collected on July 15, 2004 in a Wheaton glass bottle, taken back to the lab and refrigerated. Bacteria plates were then prepared with a 500-mL solution containing 7.5 g tryptone, 5 g yeast extract, 2.5 g sodium chloride, and 7.5 g agar. After incubating the plates for two days at 38°F, it was determined that a 45% strength by volume concentration of 2% $NaHSO_4$ would kill the bacteria in the sewage. A 1-L septic tank sample was collected on July 21,2004 and 3 separate samples were filtered for analysis to get a background concentration of caffeine and bromide in the tank, which was determined to be 0.08-ppm for the caffeine and no response was detected for the bromide. All caffeine samples were pre-filtered using sterile Millex-HA 0.45 µm Millipore syringe driven filters before analysis by HPLC to determine if caffeine was present. Bromide samples were pre-filtered using sterile Millex-GV 0.22 µm Millipore syringe driven filters to determine the bromide concentration by IC.

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[1] Images in this thesis are presented in color

The field experiment was then set up on July 22, 2004 at approximately 4:50 pm by mixing a 1-ppm solution of caffeine and a 5-ppm solution of bromide together in 2-L of DDI water and introducing it as a slug input into the crock of the residence's basement by pouring it down the laundry tub drain. The faucet was kept running for approximately one hour until the first sample was taken from the septic tank at about 6:10 pm. Sewage was sampled twice daily during the week and once on the weekend until July 28. Samples were then obtained from the septic tank once per day from July 29 to August 2. The equivalent strength by mass of 0.28g of $NaHSO_4$ was added to 20-mL glass bottles with foil-lined screw caps to collect three different samples for caffeine analysis. Three different samples without any $NaHSO_4$ were collected for bromide analysis for a total of six samples per sample period.

To conduct the aerobic and anaerobic laboratory study for caffeine degradation, approximately 500-mL of sewage was collected on July 26, 2004 and was analyzed to ascertain its background caffeine concentration, which was determined to be 0.65-ppm. To create the aerobic reactor, 150-mL of this unfiltered sewage was place in a stoppered Erlenmeyer flask on the same day with a constant supply of oxygen from a fish tank aerator. The anaerobic reactor was prepared the same day under an anaerobic hood with 150-mL of the unfiltered sewage in a 160-mL volume serum bottle and crimped with a rubber stopper. After removal from the hood after preparation, the anaerobic reactor was placed on a platform shaker until a sample was taken so that both the aerobic and anaerobic reactors simulated continuously stirred tank reactors (CSTRs). Samples were taken once per day from both reactors for six days until August 2, 2004.

RESULTS AND DISCUSSION

As stated in the above section the concentration of caffeine in the septic tank before beginning this study was about 0.08-ppm. After adding a 1-ppm slug of caffeine into the tank and monitoring the concentration for eleven days the concentration of caffeine peaked at 1.74-ppm roughly 4 hours after the experiment was began on the first day. The caffeine concentration then decreased rather quickly and nearly returned to its background concentration with a value of 0.14-ppm measured on the last day of the study[2]. The measured caffeine concentration in the septic tank may have been higher than the 1-ppm caffeine slug input after 4 hours if the slug concentration was actually slighter higher than 1-ppm or higher than normal inputs of caffeine to the septic tank from the household on that day could have contributed to the slug input.

Sewage water was tested before adding the 5-ppm bromide slug and no response was detected. Therefore, it can be assumed that the background bromide concentration in the septic tank was below the detection limit of the IC. The bromide concentration remained fairly constant at about 5.3-ppm for 24 hours after it reached the septic tank on the first day it was added. It then began to decrease at a much slower rate than the caffeine and had only been reduced to a concentration of 2.1-ppm in the tank by the last day of the study. All concentrations reported are the average of three measurements taken at the same time. Both the caffeine and bromide concentration versus time response curves are shown below in Figure 3. A plot of the ratio of caffeine: bromide versus time is also shown in Figure 4.

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[2] See Appendix B for caffeine and bromide calibration curves and raw data

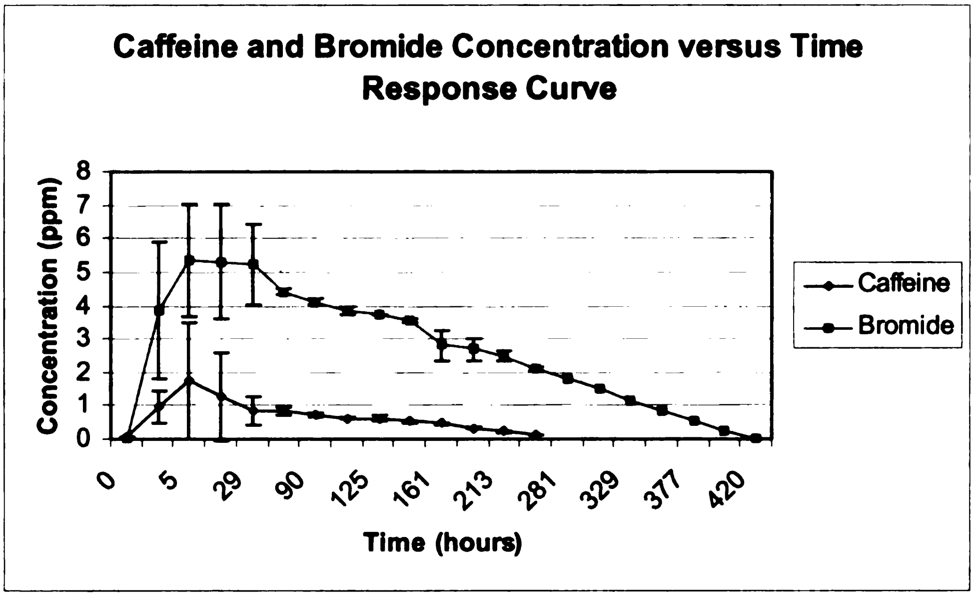

Figure 3 Caffeine and bromide concentration versus time response curve from septic tank sampling.

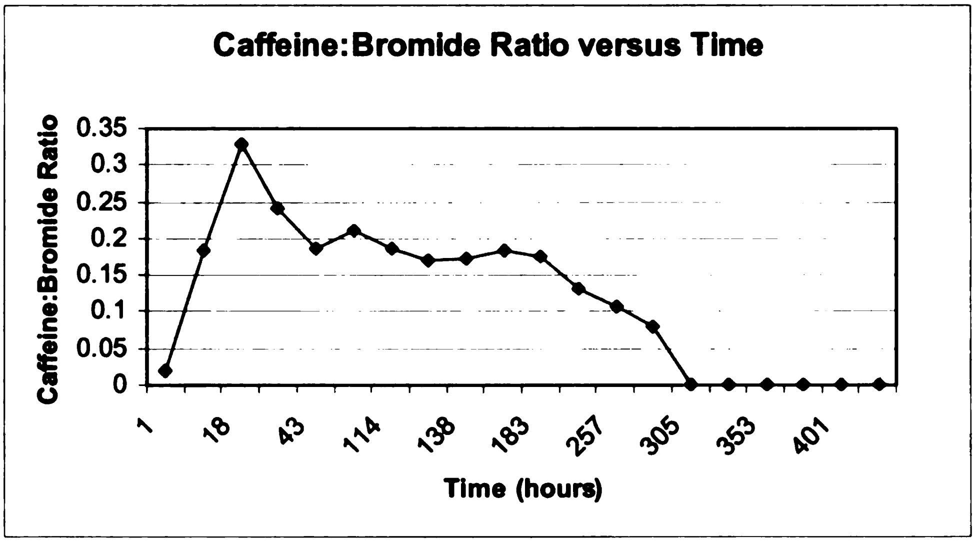

Figure 4 Caffeine to bromide ratio versus time from septic tank sampling.

Since the experiment ended after day 11 (257 hours) and the bromide concentration had not yet reached its original concentration, it was necessary to estimate the time it took for the tracer concentration to reach zero. The time required for the bromide concentration to return to zero was needed so that a hydraulic residence time could be calculated, which made it possible to calculate the approximate flow within the septic tank. This was done by averaging how much tracer was being removed from the tank per hour based on the concentration remaining in the tank after 257 hours. This hydraulic residence time was estimated to be approximately 419 hours. The flow rate using this time was calculated to be 3.57 gal/hour (85.7 gal/day).

The bromide tracer was also used to determine the amount of dispersion taking place in the tank, which would be used to determine a degradation rate for caffeine. It was assumed for this experiment that caffeine degradation followed first-order kinetics and the concentration of slug inputs for bromide and caffeine would demonstrate plug-flow with axial dispersion conditions. The following equations adopted from Metcalf and Eddy (2003) were used to calculate mean residence time, and variance values:

$$\text{Mean residence time, } t_{\Delta c} \approx \frac{\Sigma t_i C_i \Delta t_i}{\Sigma C_i \Delta t_i} \qquad \text{Variance, } \sigma_{\Delta c}^{2} \approx \frac{\Sigma t_i^{2} C_i \Delta t_i}{\Sigma C_i \Delta t_i} - (t_{\Delta c})^{2}$$

where ti is time at *i*th measurement in hours, Ci is the concentration at *i*th measurement in ppm (mg/L), Δti is the time increment about Ci in hours, and $\sigma\Delta c^2$ is the variance based on discrete time measurements in (hours)^2. For this experiment the mean residence time was 111.5 hours and the variance was 102.1 hours. A dispersion number and dispersion coefficient of 0.03 and 2.19×10^{-2} ft^2/hr respectively[3], were calculated from the equation below also adopted from Metcalf and Eddy (2003):

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[3] See Appendix C for complete tables of calculations for residence time, flow rate, and dispersion

$$\sigma_{\Delta\theta}^2 = \frac{\sigma_{\Delta c}^2}{\tau^2} \approx \frac{2\,D}{uL} = 2d$$

where τ is the hydraulic residence time in hours, D is the coefficient of axial dispersion in ft^2/day, u is fluid velocity in ft/day , L is the characteristic length in ft, and d is the dispersion number, unitless. The above equations were all based upon equations for plug-flow reactors with small amounts of axial dispersion of a conservative tracer.

Finally, the degradation of caffeine was determined. Thirumurthi (1969) as reported in Metcalf and Eddy (2003) developed a graph of kτ versus percent remaining (C/C_0) for various dispersion numbers and first-order kinetics. This plot was used to interpolate a kt value of caffeine for d = 0.03 and is presented below in Figure 5. Following this scheme a reaction rate constant (k) of 0.0064 mg/L-hr (0.154 mg/L-day) was obtained for caffeine degradation in the septic tank[4].

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[4] See Appendix D for detailed calculations of k in the septic tank

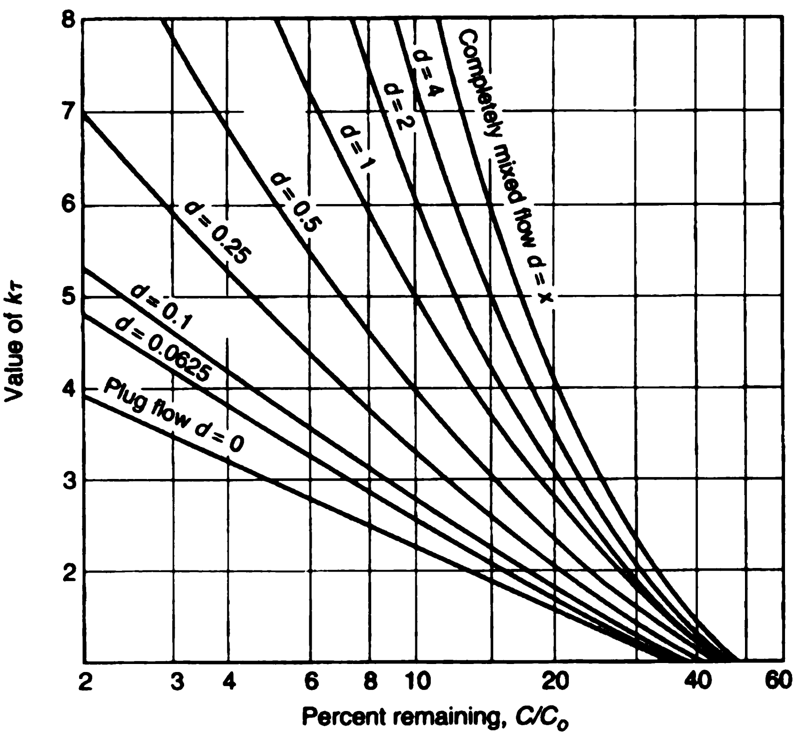

Figure 5 Graph of kτ versus percent remaining, C/C_0 for first-order kinetics (Metcalf and Eddy, 2003).

The aerobic reactor demonstrated a very rapid rate of degradation with the concentration of caffeine decreasing from 0.65-ppm to 0.18-ppm in just 17 hours. In less than 2 days, (40 hours) no caffeine could be detected in the reactor. The anaerobic reactor demonstrated very different results and the overall concentration of caffeine did not change. In fact, caffeine concentrations of 0.68-ppm and 0.75-ppm that were measured on day one and day six respectively, were actually slightly higher than the original concentration of 0.65-ppm. The aerobic degradation rate for the laboratory reactor was achieved using the equation for a continuously stirred batch reactor with a first-order rate reaction: $C/C_0 = e^{\wedge}(-k\tau)$ where C is the effluent concentration in ppm, C_0 is the influent concentration in ppm, k is the first-order reaction constant (per hour), and τ is the hydraulic residence time in hours. The aerobic degradation rate[5] was found to be 0.077 mg/L-hour (1.834 mg/L-day). However, an anaerobic degradation rate could not be determined since the final measured concentration of the anaerobic reactor was greater than the initial concentration. Therefore, it can be said that there was no significant degradation of caffeine in the anaerobic reactor. The concentration versus time response curves for both reactors is shown below in Figure 6. All caffeine and bromide calculations were plotted according to a 95% confidence level assuming sampling from a normal population for a small value of n (n < 30).

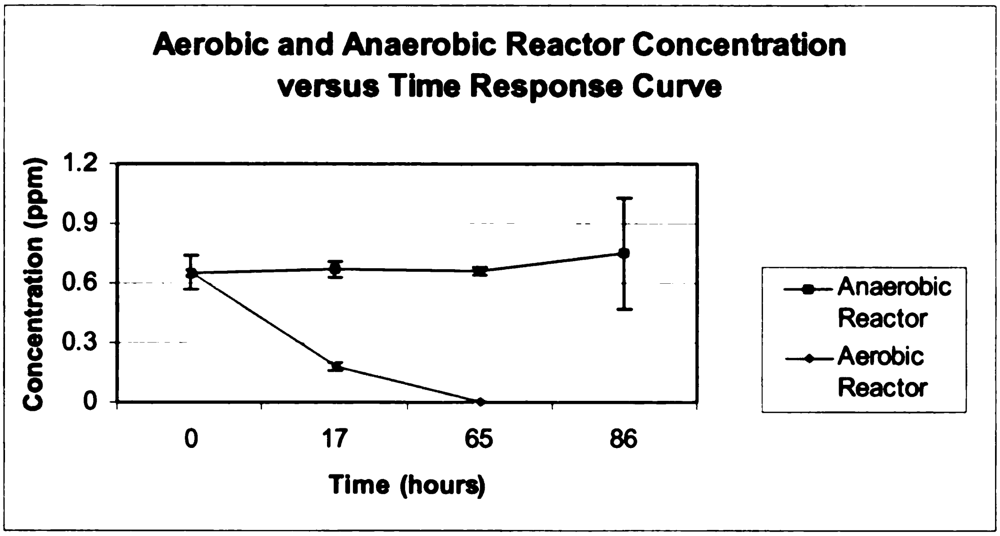

Figure 6 Aerobic and anaerobic reactor concentration versus time response curve from laboratory batch reactors.

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[5] See Appendix E for detailed calculations of k in the aerobic and anaerobic reactors

CONCLUSIONS

It appears that anaerobic degradation of caffeine is indeed significantly slower than aerobic degradation by at least one order of magnitude as shown by the vastly different rates for the septic tank and aerobic reactor. Yet, an explicit comparison could not be made between the aerobic and anaerobic reactor degradation rates because of the ineffectiveness of the anaerobic reactor in the laboratory. It was also thought that the anaerobic degradation rate in the septic tank and the anaerobic degradation rate in the lab could be compared to show the effects of plug-flow dispersion on the rate of degradation in the tank. However, the anaerobic reactor in the lab did not seem to exhibit any characteristics of constant degradation. One of the major factors contributing to this could be that the concentrations of caffeine-degrading bacteria in the laboratory septic tank sample were not large enough to promote degradation. Concentrations of caffeine that were larger than the background concentration were likely due to sampling or analysis error.

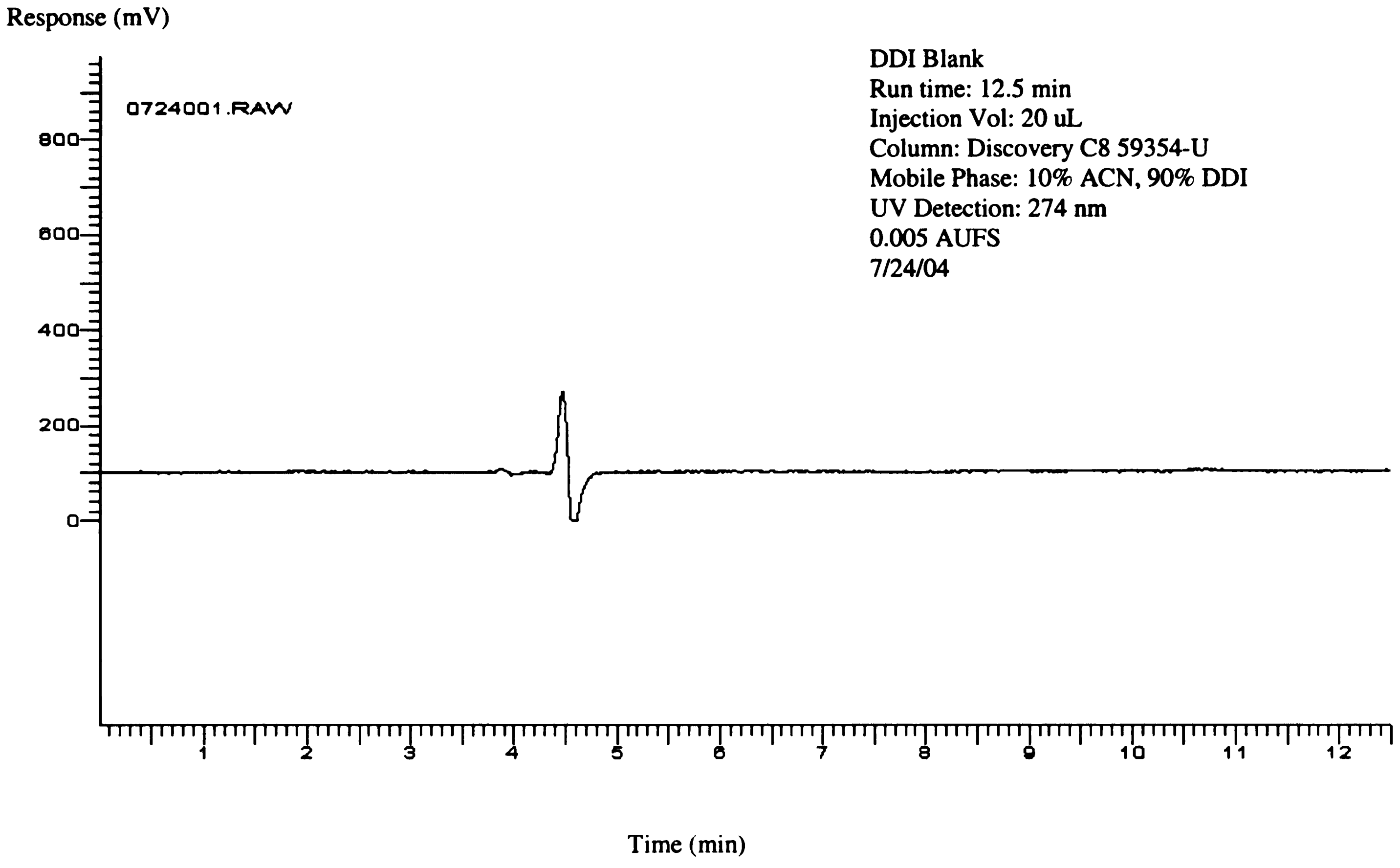
The assumptions made during this investigation that caffeine degradation in the septic tank would follow first-order kinetics and that small amounts of plug-flow dispersion would be observed, seem to be correct. For example, the dispersion number of 0.03 calculated for this study was less than 0.05. According to Metcalf and Eddy (2003), dispersion numbers less than 0.05 are characteristic of low dispersion. This low dispersion number shows that dispersion within the tank does not have a large effect on caffeine degradation. Bacterial degradation of caffeine in the septic tank also plays a minor role in its degradation as shown by the concentrations of caffeine in the laboratory anaerobic reactor remaining almost constant. All of these factors show that aerobic processes are likely to be the driving force behind caffeine degradation.

Although the existence of caffeine at a suspected crime scene does not assign specificity to a particular person, the fact that caffeine degrades fairly slowly under anaerobic conditions including when daily flushing of the system occurs, shows that caffeine can aide forensic investigators in determining possible human activity at the scene within several days of the activity. If a method can be established for the determination of caffeine metabolites in water, there are even further implications of this study. For example, an approximation of the last time that there was human activity in the area with respect to days, weeks, etc. can be provided if ratios of caffeine remaining versus the current metabolite concentration can be determined. The preliminary study completed during this experiment of measuring caffeine concentrations in the Red Cedar River also suggests that detection of caffeine in aerobic environments could be helpful in forensic studies. Suppose a person commits a crime and then escapes to a wooded or forested area to avoid being caught. In the event that this area has a stream, river, or other type of surface water and the suspect has been drinking coffee or using other products containing caffeine, the caffeine will most likely show up in the water. Large amounts of caffeine such as that from dumping coffee or rinsing coffee pots in the water would definitely be detected if the water were tested for caffeine, indicating human activity in the area. Again, caffeine ratios of caffeine remaining to the amount of metabolite will offer information on the last time humans were in the area and can give investigators and idea of how near to the area the suspect may still be.

This study is not only limited to coffee since caffeine from other sources such as caffeinated soft drinks, medications, and some foods, including chocolate, will also be detected in an anaerobic or aerobic water sample if tested for caffeine. After further testing and investigation, perhaps caffeine can be used in conjunction with other forensic analytical tools as an environmental tracer in anaerobic as well as aerobic locations. Similar studies such as this one would have to be repeated to verify the validity of caffeine as a human environmental tracer as well as studies to gather average or background concentrations of caffeine in septic tanks or similar environments. Future research may need to address other components in human waste that may alter the fate of caffeine in septic tanks or other anaerobic environments and to establish analytical methods for the testing of caffeine metabolites in aquatic environments.

APPENDIX A

Caffeine Calibration Chromatograms



DDI Blank
Run time: 12.5 min
Injection Vol: 20 uL
Column: Discovery C8 59354-U
Mobile Phase: 10% ACN, 90% DDI
UV Detection: 274 nm
0.005 AUFS
7/24/04

Figure 7 DDI Blank.

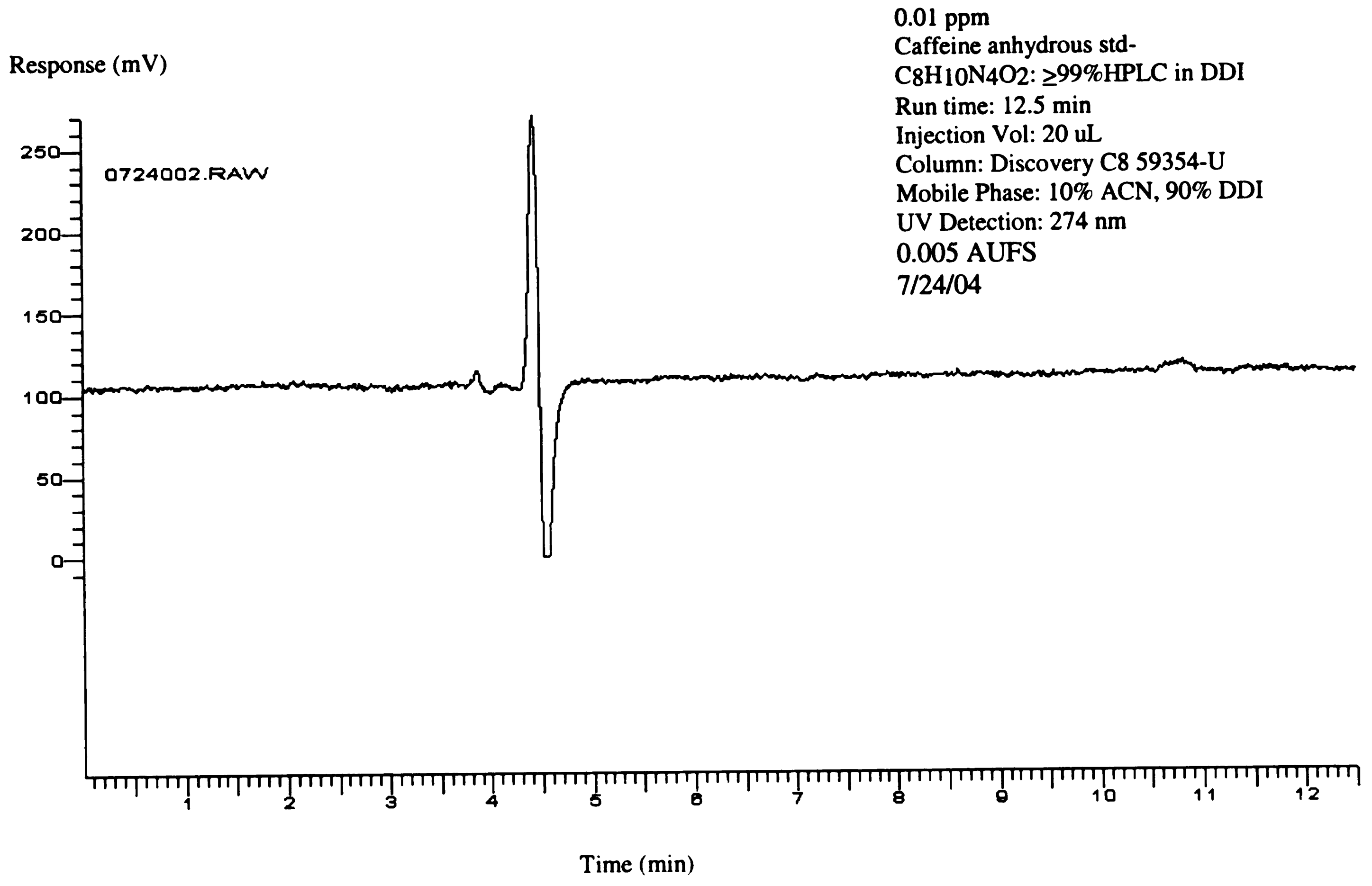
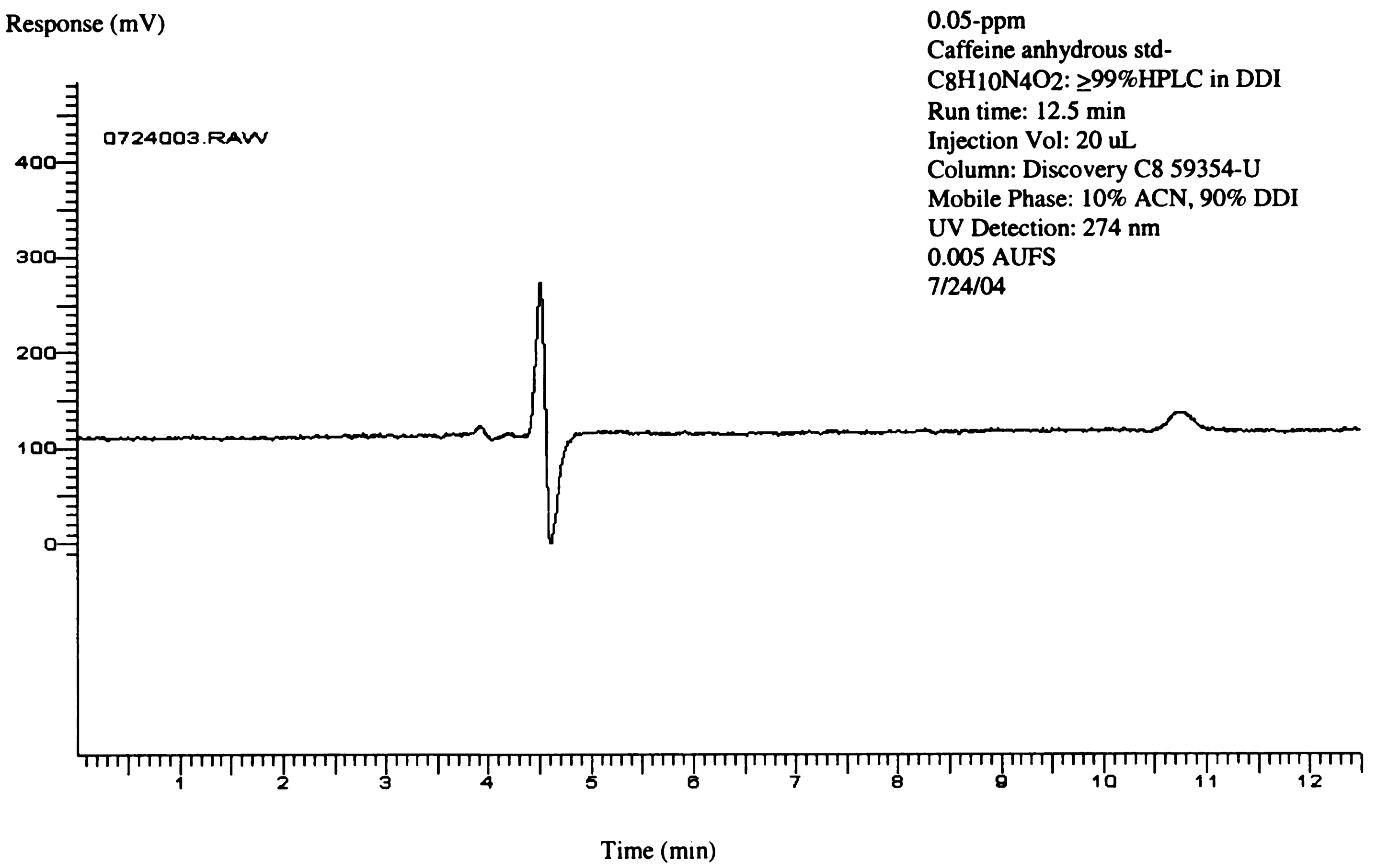


Figure 8 Caffeine standard – 0.01-ppm.



0.05-ppm
Caffeine anhydrous std-
C8H10N4O2: ≥99%HPLC in DDI
Run time: 12.5 min
Injection Vol: 20 uL
Column: Discovery C8 59354-U
Mobile Phase: 10% ACN, 90% DDI
UV Detection: 274 nm
0.005 AUFS
7/24/04

Figure 9 Caffeine standard – 0.05-ppm.

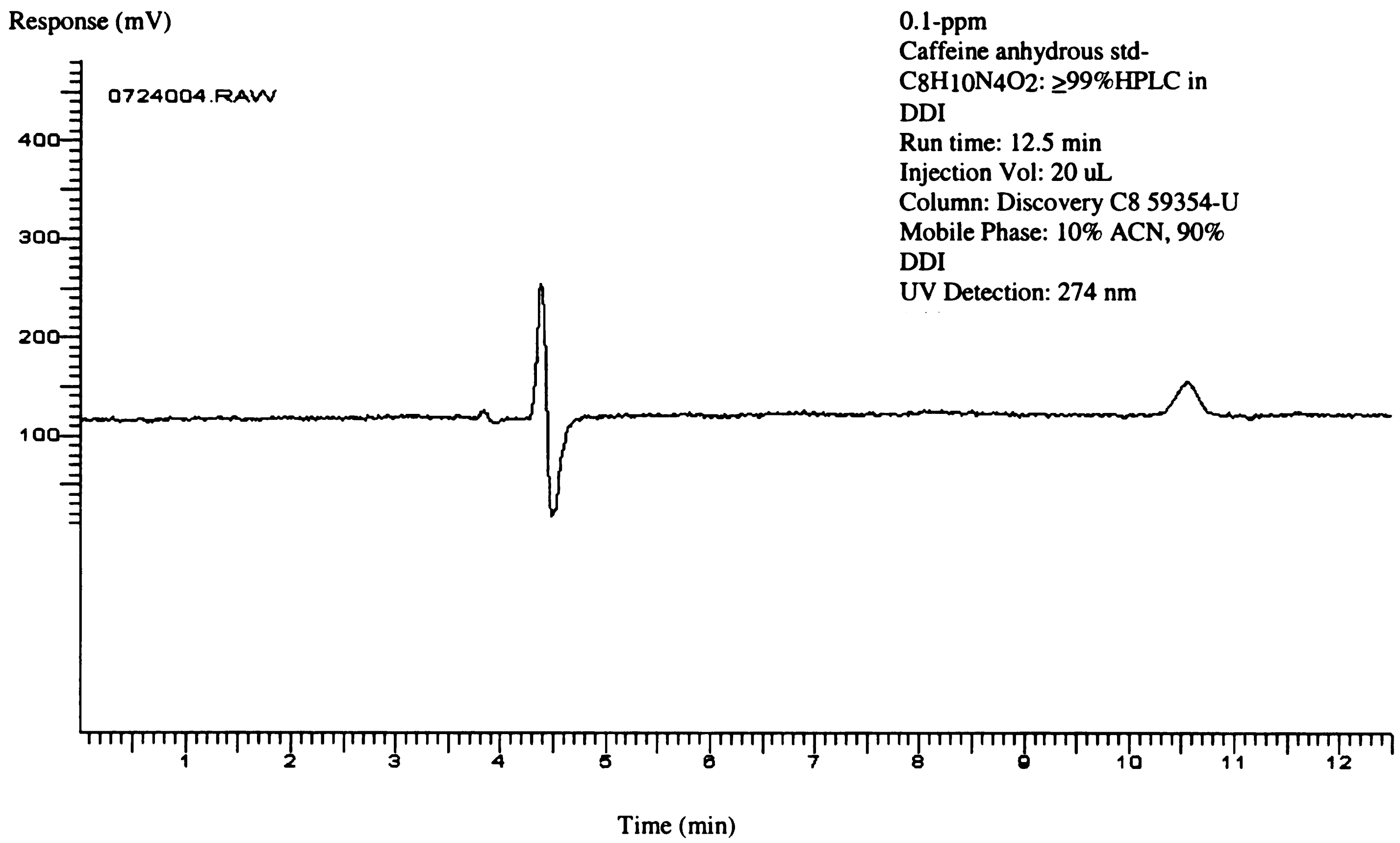


Figure 10 Caffeine standard – 0.1-ppm.

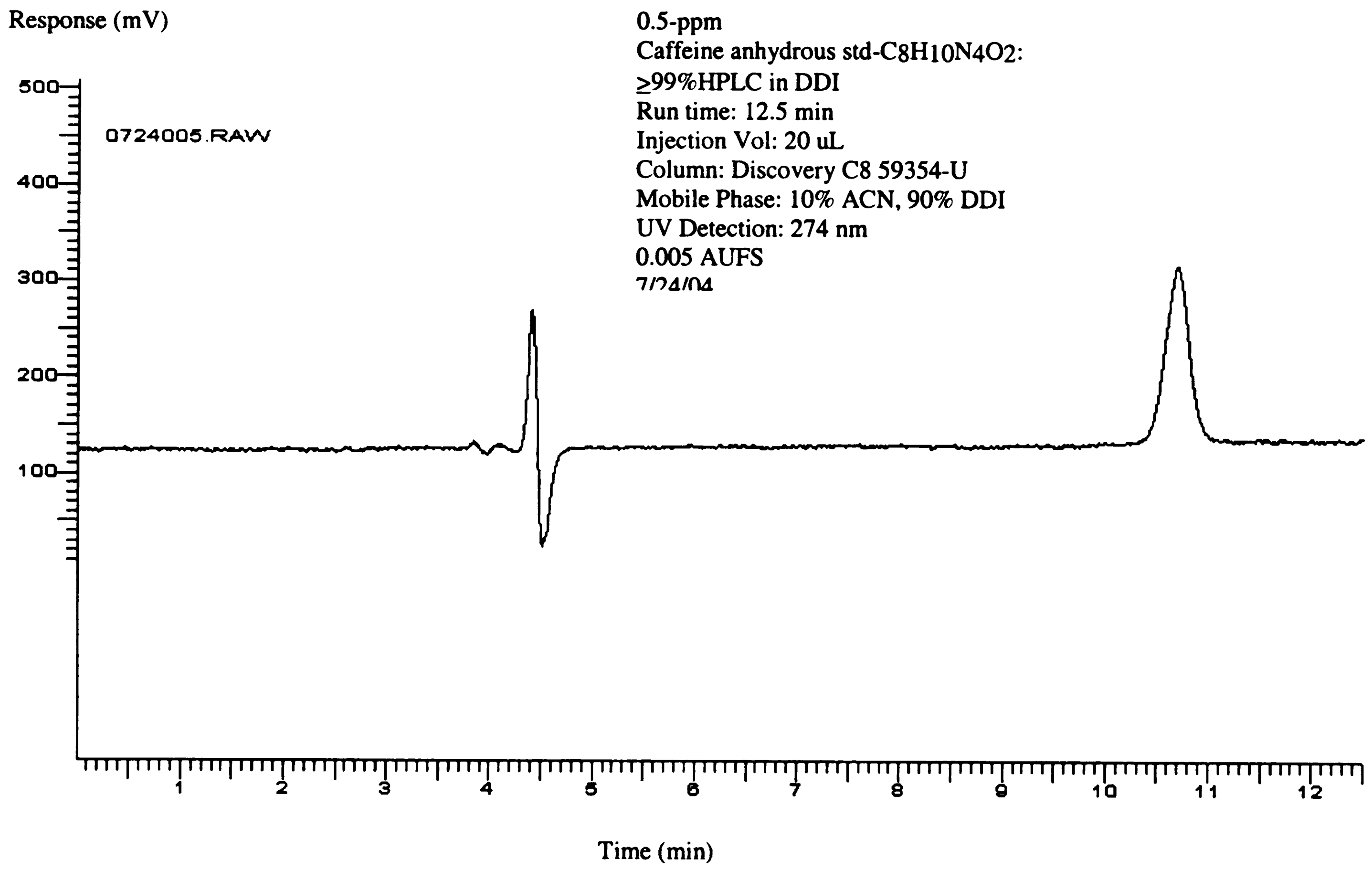
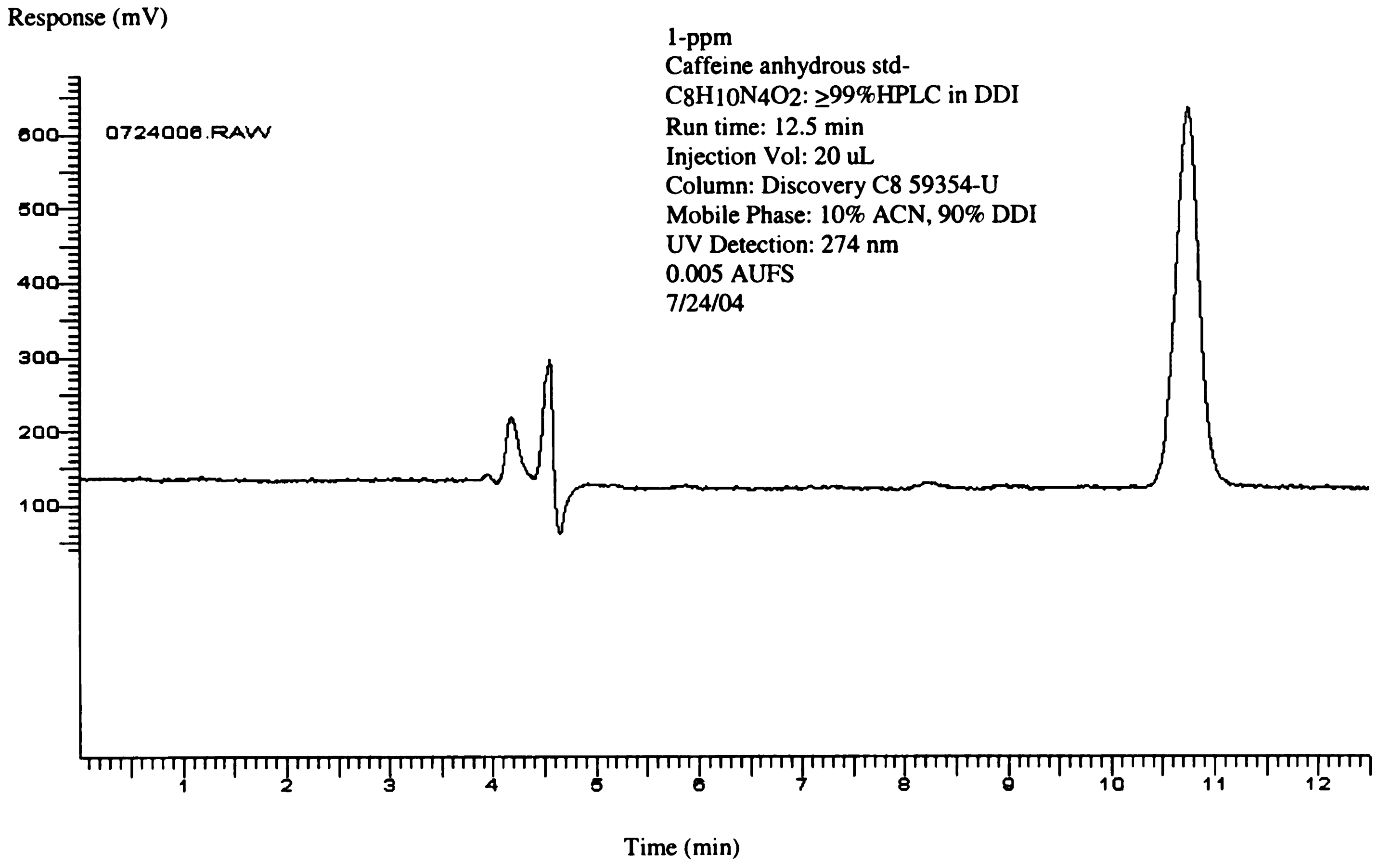


Figure 11 Caffeine standard – 0.5-ppm.



1-ppm
Caffeine anhydrous std-
$C_8H_{10}N_4O_2$: ≥99%HPLC in DDI
Run time: 12.5 min
Injection Vol: 20 uL
Column: Discovery C8 59354-U
Mobile Phase: 10% ACN, 90% DDI
UV Detection: 274 nm
0.005 AUFS
7/24/04

Figure 12 Caffeine standard – 1-ppm.

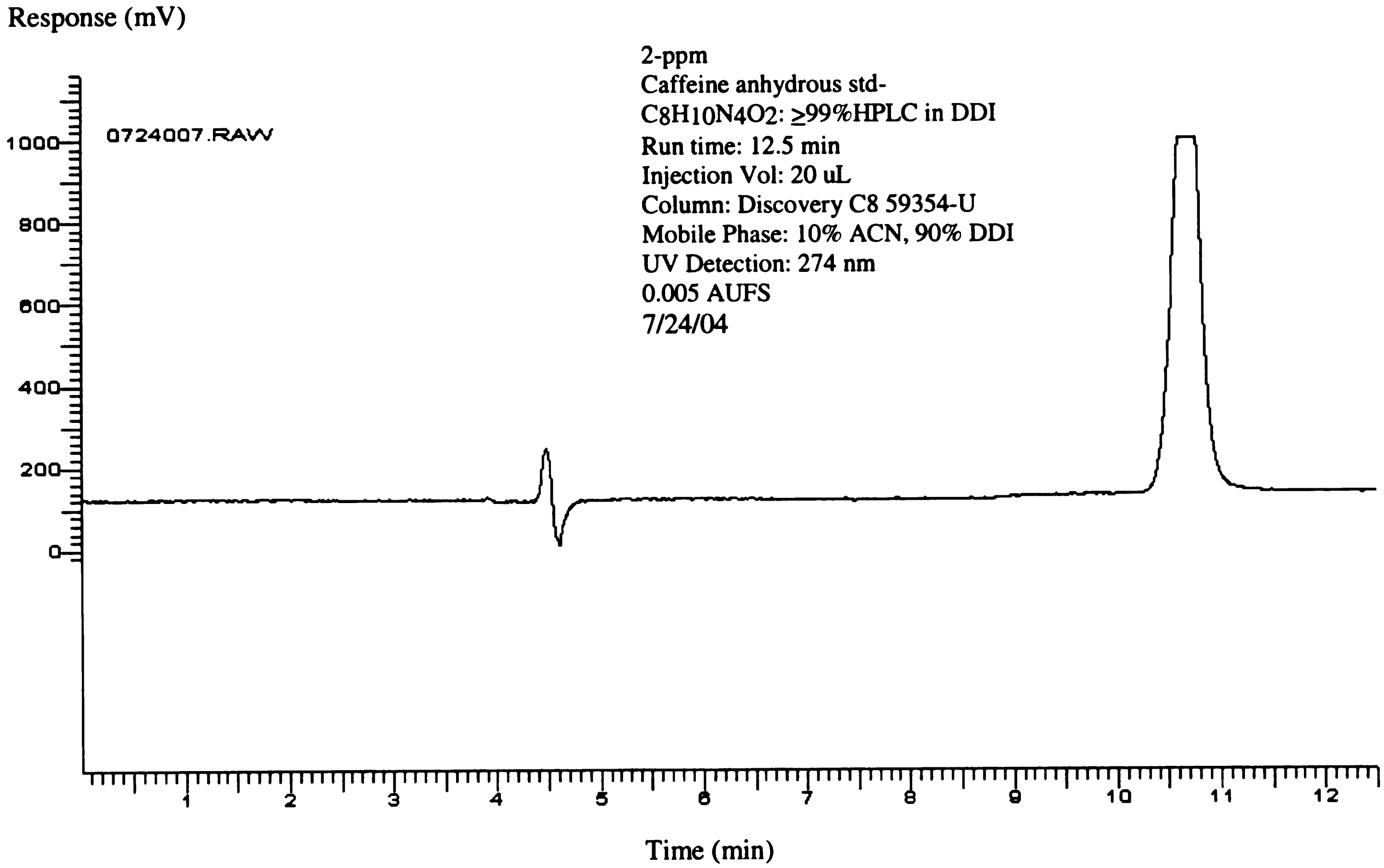


Figure 13 Caffeine standard – 2-ppm.

APPENDIX B

Caffeine and Bromide Calibration Curves and Raw Data

Table 1 Caffeine Calibration Data

Caffeine Calibration Data	
ppm	**Response**
0.01	71303.13
0.05	287789.33
0.1	429950.85
0.5	2915290.24
1	7873548.41
2	18351864.41

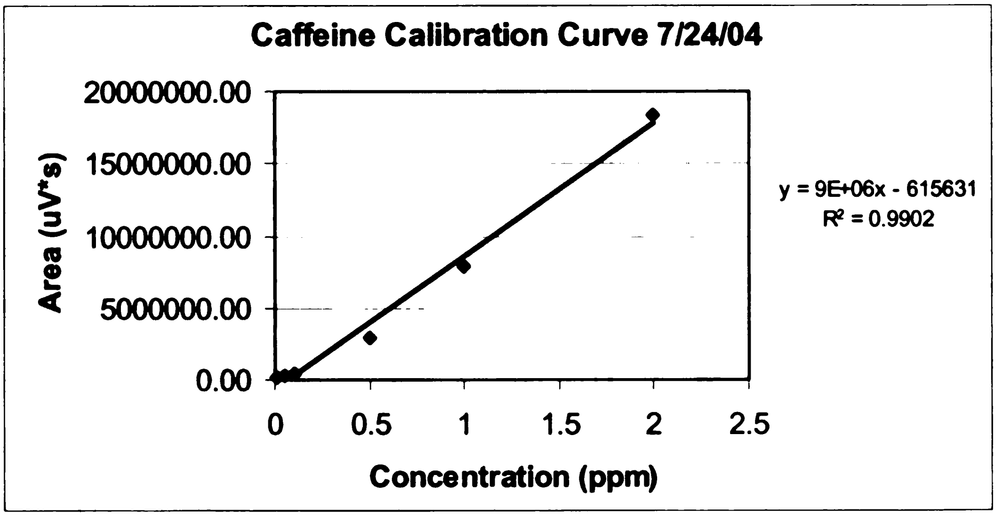


Figure 14 Caffeine calibration curve.

Table 2 Bromide Calibration Data

Bromide Calibration Data	
ppm	**Response**
1	29159
2	102988
5	343180
10	839221

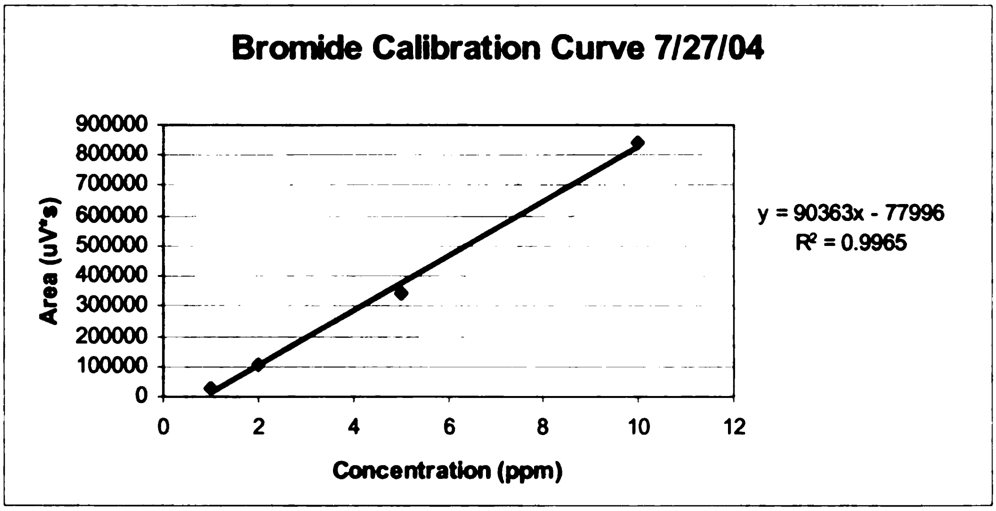


Figure 15 Bromide calibration curve.

Table 3 Caffeine Raw Data

Caffeine Raw Data							
Date	**Time**	**Conc. (ppm)**	**Response**	**n**	**Std Dev**	**tinv**	**Confidence Interval**
7/21/04	1:30 PM	0.077	78873.39				
		0.079	93832.48				
		0.078	82645				
		0.078	**85116.96**	3	0.0009	4.302656	0.00263
After Slug Input							
7/22/04	6:10 PM	0.813	6700468				
		1.135	9601250				
		0.969	8103624				
		0.972	**8135114**	3	0.1612	4.302656	0.4903891
7/22/04	10:05 PM	1.092	9212511				
		2.140	18643552				
		1.996	17345104				
		1.743	**15067056**	3	0.568	4.302656	1.7279627
7/23/04	10:35 AM	1.090	9194176				
		1.748	15113799				
		0.939	7839496				
		1.259	**10715824**	3	0.4298	4.302656	1.3077469
7/23/04	10:00 PM	0.881	7316261				
		0.667	5387880				
		0.933	7781688				
		0.827	**6828610**	3	0.141	4.302656	0.4290598
7/24/04	11:50 AM	0.845	6990280				
		0.840	6943134				
		0.908	7556916				
		0.864	**7163443**	3	0.038	4.302656	0.1154679
7/26/04	10:50 AM	0.700	5687305				
		0.704	5721404				
		0.735	5995770				
		0.713	**5801493**	3	0.0188	4.302656	0.0571677
7/27/04	11:00 AM	0.631	5065710				
		0.628	5033057				
		0.621	4973552				
		0.627	**5024106**	3	0.0052	4.302656	0.0157959
7/27/04	10:00 PM	0.592	4712133				
		0.594	4730397				
		0.646	5199304				
		0.611	**4880611**	3	0.0307	4.302656	0.0933511
7/28/04	10:50 AM	0.514	4011476				
		0.523	4087329				
		0.518	4047109				
		0.518	**4048638**	3	0.0042	4.302656	0.0128287
7/29/04	10:30 AM	0.463	3554362				
		0.455	3475212				
		0.482	3720580				
		0.467	**3583384**	3	0.0139	4.302656	0.0423347
7/30/04	8:30 AM	0.333	2384901				
		0.318	2249970				
		0.329	2345442				

Table 3 (cont'd)

Date	**Time**	**Conc. (ppm)**	**Response**	**n**	**Std Dev**	**tinv**	**Confidence Interval**
		0.327	**2326771**	3	0.0077	4.302656	0.0234525
7/31/04	2:30 PM	0.224	1399831				
		0.213	1299855				
		0.235	1499450				
		0.224	**1399712**	3	0.0111	4.302656	0.0337364
8/2/04	10:35 AM	0.146	698958.2				
		0.143	674825.2				
		0.144	679869.9				
		0.144	**684551.1**	3	0.0014	4.302656	0.0043031

Table 4 Bromide Raw Data

Bromide Data							
Date	**Time**	**Conc. (ppm)**	**Response**	**n**	**Std Dev**	**tinv**	**Confidence Interval**
7/22/04	6:10 PM	3.176	209042				
		4.535	331844				
		3.884	272953				
		3.865	**271279.7**	3	0.679682	4.302656	2.067889
7/22/04	10:05 PM	5.124	385055				
		5.962	460772				
		4.929	367363				
		5.338	**404396.7**	3	0.54909	4.302656	1.670571
7/23/04	10:35 AM	5.732	439944				
		4.676	344543.5				
		5.527	421480				
		5.312	**401989.2**	3	0.55995	4.302656	1.703612
7/23/04	10:00 PM	4.802	355903.5				
		5.557	424125.5				
		5.373	407529.5				
		5.244	**395852.8**	3	0.393727	4.302656	1.197889
7/24/04	11:50 AM	4.408	320321.5				
		4.462	325221.5				
		4.390	318687				
		4.420	**321410**	3	0.037632	4.302656	0.114492
7/26/04	10:50 AM	4.132	295420				
		4.109	293309				
		4.071	289907				
		4.104	**292878.7**	3	0.030782	4.302656	0.093653
7/27/04	11:00 AM	3.844	269374.5				
		3.891	273580				
		3.830	268130				
		3.855	**270361.5**	3	0.031605	4.302656	0.096156
7/27/04	10:00 PM	3.715	257747.5				
		3.713	257477				
		3.711	257332				
		3.713	**257518.8**	3	0.002334	4.302656	0.007100
7/28/04	10:50 AM	3.553	243054				
		3.524	240413.5				
		3.536	241495				
		3.537	**241654.2**	3	0.01469	4.302656	0.044693
7/29/04	10:30 AM	2.851	179668.5				
		2.944	188018.5				
		2.652	161624.5				
		2.816	**176437.2**	3	0.149292	4.302656	0.454210
7/30/04	8:35 AM	2.796	174688				
		2.563	153631.5				
		2.677	163865				
		2.679	**164061.5**	3	0.116526	4.302656	0.354523
7/31/04	2:30 PM	2.535	151096				
		2.452	143573.5				
		2.441	142623.5				
		2.476	**145764.3**	3	0.051368	4.302656	0.156283

Table 4 (cont'd)

Date	**Time**	**Conc. (ppm)**	**Response**	**n**	**Std Dev**	**tinv**	**Confidence Interval**
8/2/04	10:35 AM	2.103	112050.5				
		2.106	112346.5				
		2.075	109550				
		2.095	**111315.7**	3	0.017001	4.302656	0.051724

APPENDIX C

Bromide Dispersion Calculations

Table 5 Bromide Tracer Data

BROMIDE TRACER DATA				
		Time (hrs)	**Conc (ppm)**	
		0	0	
		1.33	3.85	
		5.25	5.338	
		17.75	5.312	
		29.17	5.244	
		43	4.42	
		90	4.104	
		113.83	3.855	
		124.83	3.713	
		137.67	3.537	
		161.33	2.816	
		183.42	2.679	
		213.33	2.476	
		257.42	2.095	
Interpolated values		281.42	1.786351073	
based on how long		305.42	1.477702073	
it took to reduce		329.42	1.169053073	
conc from 5.338 to		353.42	0.860404073	
2.095 ppm		377.42	0.551755073	
		401.42	0.243106073	
	t	419.92	0.005189073	
		🟦	estimated Concentrations	
Time Br is in tank (hrs)	**How much Br decreases in 252.17 hrs**	**How much Br decreases per hour**	**How much Br would decrease in 24 hrs**	**How much Br would decrease in 18.5 hrs**
252.17	3.243	0.012860372	0.308648927	0.237917
Q* (gal/hr) V/t	**Q (gal/day) V/t**			
3.572108973	85.73061536			
*based on time it took for Br to decrease to 0				

Table 6 Bromide Dispersion Data

BROMIDE DISPERSION DATA			
Time (hrs)	**Conc, C (ppm)**	**t*C**	**t^2*C**
0.00	0.000	0.00	0.00
1.33	3.850	5.13	6.84
5.25	5.338	28.02	147.13
17.75	5.312	94.29	1673.61
29.17	5.244	152.97	4462.06
43.00	4.420	190.06	8172.58
90.00	4.104	369.36	33242.40
113.83	3.855	438.81	49950.27
124.83	3.713	463.49	57857.93
137.67	3.537	486.94	67036.86
161.33	2.816	454.31	73293.07
183.42	2.679	491.38	90129.32
213.33	2.476	528.21	112681.99
257.42	2.095	539.29	138825.29
281.42	1.786	502.71	141474.03
305.42	1.478	451.32	137842.08
329.42	1.169	385.11	126862.76
353.42	0.860	304.08	107469.37
377.42	0.552	208.24	78595.22
401.42	0.243	97.59	39173.63
419.92	0.005	2.18	915.00
Σ	**55.533**	**6193.51**	**1269811.47**
🟦	estimated Concentrations		
Mean residence time, $t_{\Delta c}$ (hrs)	**Σ t2*C/Σ C**	**Σ t2*C/Σ C - $(t_{\Delta c})^2$, $\sigma^2_{\Delta c}$, (hrs^2)**	**Variance, $\sigma_{\Delta c}$ (hrs)**
111.53	22866.071	10427.29	102.11
Q ft^3/hr	**Vol ft^3**	**Length ft**	**Area ft^2**
0.47838	201	13.4	67
τ, (hrs)	**Dispersion No.**	**Velocity**	**Dispersion factor (ft^2/hr)**
419.92	0.03	7.35E-02	2.91E-02
Assumptions:			
plug flow dispersion			
first-order kinetics			
Q can be estimated by using volume of tank and estimation of time tracer is in reactor			

APPENDIX D

Detailed k Calculation for Septic Tank

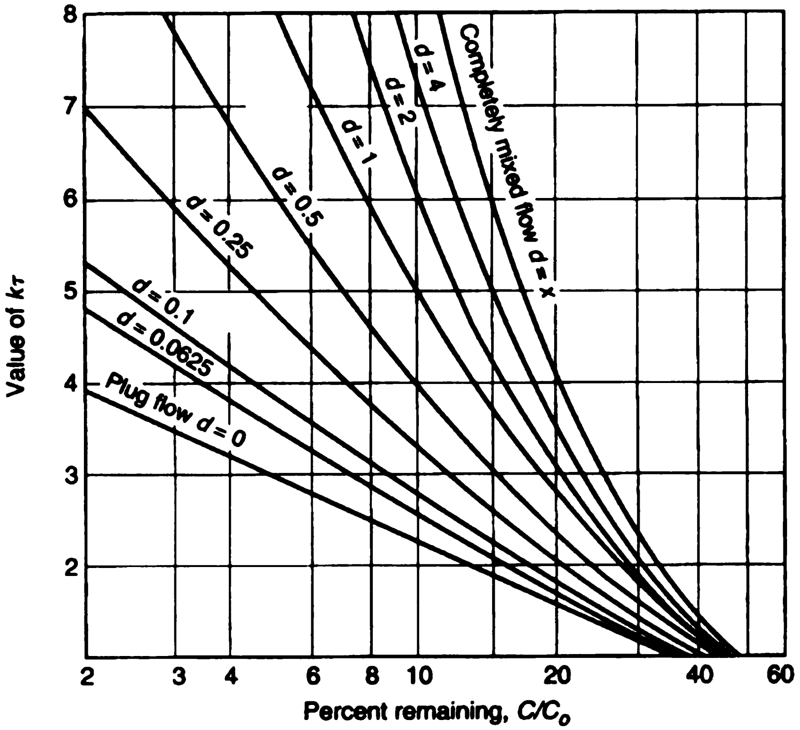


Figure 16 Graph of kτ versus percent remaining, C/C_0 for first-order kinetics (Metcalf and Eddy, 2003).

From the above graph by Thirumurthi (1969) as reported in Metcalf and Eddy (2003), kτ values for d = 0 and d = 0.0625 were read. These values and the corresponding C/Co value for the tank were used to interpolate what kτ would be for d = 0.03 (the dispersion number of d = 0.03 was calculated using the bromide data and can be found in Appendix A).

1. Calculate C/Co for tank:
   Final C = 0.144 and Co after adding tracer = 1.743, C/Co = 8.261

2. Read kτ for d = 0 and C/Co = 8.3
   kτ = 2.5

3. Read kt for d = 0.0625 and C/Co = 8.3
   kτ = 2.9

4. Interpolate kτ for d = 0.03 using:

$$\frac{0.0625 - 0.03}{0.0625 - 0} = \frac{2.9 - x}{2.9 - 2.5}$$

5. (0.0625-0.03)*(2.9-2.5) = 0.013
6. (2.9*0.0625) – 0.013 = 0.16825
7. 0.16825/0.0625 = 2.692 = x = $k\tau$
8. Recall that for the tank the residence time, τ = 419 hours
9. Therefore, k for the tank = 2.692/419 hrs = 0.0064/ hr
   0.0064/ hr*(24 hrs/day) = 0.154/day

APPENDIX E

Detailed k Calculation for Laboratory Reactors

Table 7 Aerobic Reactor Data

Date	Time (hours)	Concentration (ppm)
7/26/04	0	0.654
7/27/04	17	0.178
7/28/04	40	0

Aerobic Reactor - Initial Setup: Monday July 26, 2004 5:25 pm

$C/C_0 = e$^$(-k\tau)$

k = (ln(0.178/0.654))/(-17) = 0.077mg/L-hr (Aerobic Degradation Rate)

0.077/hr x 24 hr/day = 1.84mg/L-hr

Table 8 Anaerobic Reactor Data

Date	Time (hours)	Concentration (ppm)
7/26/04	0	0.654
7/27/04	17	0.667
7/29/04	65	0.661
8/2/04	86	0.686

Anaerobic Reactor - Initial Setup: Monday July 26, 2004 5:30 pm

$C/C_0 = e$^$(-k\tau)$

k = (ln(0.75/0.654))/(-86) = -0.002mg/L-hr ≈ 0 (Anaerobic Degradation Rate)

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