

Single Drop Microextraction: An Educational Undergraduate Laboratory Procedure Using

Caffeine and DEET

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ABSTRACT

Single drop microextraction (SDME) is applicable for the analysis of caffeine and DEET in aqueous samples in an undergraduate laboratory. A drop of organic solvent (toluene or chloroform) is suspended in stirred aqueous solution in order to extract the desired analyte. The drop is injected into a gas chromatograph mass spectrometer in single ion monitoring (SIM) mode. The chromatograms are integrated to construct a calibration curve using the peak areas. The following variables were optimized: drop size (0.5 microliters), stirring rate (200 rotations per minute), sample volume (4 milliliters), extraction time (5 minutes), and chloroform as the organic extraction solvent. When caffeine was extracted into chloroform the percent relative standard deviation of the peak areas and detection limit were 2 to 38% and 1.1×10^{-3} mg/mL. The caffeine extracted into chloroform showed good linearity with a correlation coefficient of 0.995. Samples of Diet Coke, Barq's Root Beer, Xenergy energy drink, Twinings Lady Grey Tea, and Stash Vanilla Chai Decaf Tea were all prepared and analyzed for caffeine content in comparison to reported values. DEET (N,N-diethyl-3-methylbenzamide) extracted into chloroform had a percent relative standard deviation and detection limit of 2 to 64% and 6.6×10^{-3} mg/mL. The DEET extracted into chloroform showed good linearity as well, with a correlation coefficient of 0.960. Internal standards were not successfully applied to the extractions with either caffeine, DEET, or para-xylene. Other adaptations of SDME include the analysis of ethanol in mouthwash and phenol in Chloraseptic® spray. Overall, the procedure is easily reproduced and requires equipment and solvents found in most undergraduate laboratories. Students were able to complete the procedure with relative ease and good reproducibility.

INTRODUCTION

Extractions.

Liquid-liquid extraction is used in analytical chemistry laboratories as a sample preparation step for instrumental analysis. Traditionally, liquid-liquid extraction requires large amounts of hazardous solvents, which require appropriate waste management and disposal. In order to reduce the volume of solvent required, techniques have been developed to perform extractions on a smaller scale. These general types of microextraction can be grouped into one

of three broad categories: solid phase microextraction, membrane microextraction, and miniaturized liquid-liquid extraction.¹ The purpose of this study is to examine the applications of miniaturized liquid-liquid extraction to the undergraduate educational laboratory.

Single drop microextraction (SDME) uses a drop of solvent to extract analyte from the sample solution, as developed in 1997. The microdrop minimizes the required volume of solvent. This type of microextraction may be applied to various analytical instruments and analytes with some modifications.¹

Instrumentation

For most SDME experiments, an organic solvent is used to extract the analyte from an aqueous solution. The technique lends itself to analysis with gas chromatography and mass spectrometry because no additional steps are required to prepare the organic solvent for injection into the instrument.²

Gas chromatography (GC) uses a gaseous mobile phase to pass a gaseous analyte through a column containing some type of stationary phase. The analyte is introduced into the gaseous phase at the injection point, which is kept at a high temperature in order to force the phase transition (liquid to gas). The mobile phase is inert in order to prevent interactions with the analyte and the stationary phase. The stationary phase can take many forms depending on the type of gas chromatography. The stationary phase, depending on the form, separates the analyte depending on polarity, size, charge, hydrogen bonding, or other type of affinity. Many types of detectors are available to analyze various samples. A thermal conductivity detector is common because it can universally respond to analytes.³ Other GC detectors include, but are not limited to, flame ionization detectors, electron capture detectors, and flame photometric detectors.³

Mass spectrometry (MS) analyzes the masses of atoms, molecules, and fragments of molecules. When paired with gas chromatography, it is possible to detect mass-to-charge ratios specific to the analytes of interest. An ionization source between the gas chromatograph and the mass spectrometer ionizes the analyte and passes the ions through a mass analyzer. In the model used here the mass analyzer is a quadrupole. The quadrupole creates an electric field between the four charged parallel rods. The electric field is controlled to be specific to a particular mass-to-charge ratio. Ions of any other mass-to-charge ratio are deflected and neutralized along the path of the quadrupole. The ions of interest are detected by the electron multiplier at the end of the quadrupole.³ Two modes of analysis are possible with MS: SCAN and selected ion monitoring (SIM). SCAN mode scans every mass-to-charge ratio (m/z) within a determined range. SIM is a more sensitive mode which only detects specified m/z ratios for the analyte of interest. The increased sensitivity is a result of decreasing the response to background noise, allowing for the specified m/z ratios to have a larger response.³

Modifications to SDME

Static extraction uses an organic solvent drop suspended in an aqueous solution.

Dynamic liquid-phase microextraction is another form of miniaturized liquid-liquid extraction using a microsyringe. The extraction process occurs in the syringe by repeatedly withdrawing and expelling aqueous solution into the syringe with the organic solvent in order to extract the analyte.⁴ In comparison, cycle-flow extraction uses a continuous flow of aqueous solution to pass over the organic drop and allow for extraction (Figure 1). The aqueous solution is kept flowing in the bottom of container by using tubing and a pump system to create a circular stream around the organic drop.⁵

Drop-to-drop solvent microextraction uses a similar method as direct SDME but on a slightly smaller scale. A single drop of organic solvent is suspended from the tip of a syringe into a single drop of aqueous solution spiked with analyte (Figure 3). Caffeine has been analyzed using drop-to-drop SDME with success.⁶

It is possible to attach a small bell-shaped device attached to the tip of the syringe (Figure 2). This modification provides a larger surface for the organic drop to adhere to the syringe during extraction.² In order to prepare the bell-shaped device, it is necessary to attach a piece of tubing to the end of a syringe and use a file to add texture to the end of the device, making attachment to the end of the syringe easier for a drop of larger volume. Between each trial the device must be rinsed with solvent in order to prevent contamination, especially in the ridges created by the file. The use of this device showed an improvement in detection limit and sensitivity for the analysis of some organic pollutants.⁷

Headspace single drop microextraction (HS-SDME) follows the same basic principles as traditional SDME, but the drop of organic solvent is suspended in the vapor of a volatile sample in a closed vial. The analyte is extracted from the vapor above the liquid sample, as with the analysis of volatile sulfur compounds. Compared to traditional SDME, HS-SDME is more selective for volatile compounds.⁸ Student experiments using HS-SDME have been suggested, including the analysis of mouthwash. However, the suggested procedures have not been tested with undergraduate students in a laboratory setting.⁹

The precision of SDME is improved by attaching a Chaney adapter to the microsyringe. The adapter allows for a precise amount of solvent to be withdrawn and injected with each trial. Unlike other microsyringe adapters, it is possible to set an intermediate volume to allow a drop

with a specific volume to form at the tip of the syringe while maintaining a certain volume of solvent in the syringe. The adapter increases precision by reducing the amount of variability in the drop volume possible in each step in the extraction process.¹⁰

Parameters and maintenance

The optimization of SDME requires the consideration of many parameters including extraction time, extraction solvent, size of extracting drop, stirring rate, salt concentration, temperature of solution, and temperature of the GC oven.^{11,12,13,14} The parameters have been optimized for a variety of studies including the analysis of fungicide in water and wine samples, free formaldehyde in DTP and DT vaccines, and carbamate pesticides in water samples.

The extraction time must be balanced to allow for a quantifiable amount of analyte to be extracted, but not so long that the organic drop dissolves into the aqueous sample.¹¹ Previously studied extraction times range from 5 to 30 minutes.^{11,12} For the analysis of fungicide in water and wine samples, fifteen minutes was determined to be the ideal extraction time.¹¹ The analysis of free formaldehyde in DTP and DT vaccines was optimized with an extraction time of ten minutes.¹²

The extraction solvent must be immiscible with water; otherwise the drop would not stay on the tip of the syringe as it dissolved into the aqueous solution. The extraction solvent must have some affinity for the analyte in order for extraction to occur.¹¹ Potential organic solvents include, but are not limited to, xylene, toluene, cyclohexane, hexane, isooctane, carbon tetrachloride, chloroform, and butyl acetate.^{11,12}

The drop must be large enough to extract a detectable amount of analyte but small enough to suspend the drop from the tip of the microsyringe.¹¹ Typical drop sizes ranged from

0.2 to 4.0 microliters.^{12,13,14} Previous studies have examined the extraction of caffeine from aqueous solutions using SDME and found 0.5 microliter organic drops to be the optimal size.⁹

Stirring the aqueous solution allows fresh sample to be exposed to the drop during the extraction period, increasing extraction efficiency. Stirring also prohibits the formation of a diffusion gradient around the extracting drop. Without stirring, the extraction depends on the diffusion rate of the analyte in the aqueous solution from areas of high concentration to low concentration. If the rate of sample agitation is too vigorous, the drop will dislodge from the tip of the syringe.¹¹ Typical stirring rates range from 0-1000 rotations per minute.^{11,12,13}

The addition of a salt to the aqueous solution may increase the partition coefficient of the analyte to the organic drop for some analytes.¹¹ However, the use of additional salt decreased the amount of analyte extracted into the organic drop for our analytes due to interference with the transport of the analyte into the organic drop and reduction of the diffusion rates from water to microdrop of organic solvent.¹¹ Researchers have explained this result by proposing a change in the extraction film of the organic drop due to the addition of salt to the aqueous solution. The physical changes on the outer surface of the organic drop reduce the diffusion of the analyte from the aqueous to the organic phase.¹⁴

The temperature of the sample solution has a large impact on the extraction rate; therefore it is important to keep the temperature constant throughout the procedure. The higher the temperature, the faster the extraction occurs. The temperature cannot be raised too high or the drop will begin to dissolve in the aqueous solution or the analyte will vaporize.¹²

Beyond the standard optimization parameters it is necessary to maintain certain experimental conditions in order to ensure accuracy and precision. At the beginning of each day,

the syringe should be washed with solvent in order to remove air and bubbles in the solvent drop. The vial and syringe should be kept at the same position and orientation throughout all trials, including needle depth and position in the vial. The position can be regulated with the use of clamps and stands.¹⁴

Internal standards

In many chromatography experiments, an internal standard is added to an analyte solution in order to improve the precision of the measurement of the analyte. The signal strength of the internal standard is compared to the signal strength of the analyte, therefore determining the amount of analyte present in the solution.³ The use of internal standards was an effective analytical method to pair with SDME for certain analytes. The analytical signal was determined by taking the ratio of sample to internal standard.¹⁶ Phthalate esters found in certain fragrances were best analyzed using an internal standard of nonadecane in order to correct for any variability in the injection volume.¹⁶ Nonadecane was chosen as the internal standard because it was resolved from the analyte peaks.¹⁶

Methods of quantitative analysis.

Detection limit

The detection limit is defined as the lowest amount of analyte that is detected to be different from a blank.³ It can be calculated in a few ways, depending on desired level of accuracy. The analyte is detectable at three times the standard deviation of the peak area of the lowest concentration standard divided by the slope of the calibration curve (Equation 1).³

$$detection\ limit = \frac{3s}{m} \quad (Eq. 1)$$

However, there is an enhanced ability to quantitate using the lower limit of quantitation (Equation 2), which is ten times the standard deviation of the peak area divided by the slope of the calibration curve.³

$$\text{lower limit of quantitation} = \frac{10s}{m} \quad (\text{Eq. 2})$$

If least-squares data is available for the calibration curve, the detection limit may be calculated from the y-intercept and the standard deviation in the y-intercept, s_y , (Equation 3).³

$$\text{lower limit of quantitation} = \frac{10s_y}{m} \quad (\text{Eq. 3})$$

The analysis in this study was completed using the detection limit (Equation 1), as the measure of lowest possible concentration of analyte that could be detected.

Partition coefficient

The partition coefficient, K , is an indicator of extraction efficiency as an analyte transitions from one phase to another. It is defined as the ratio of the activity, a , between two phases, 1 and 2, (Equation 4).

$$K = \frac{a_{\text{organic}}}{a_{\text{aqueous}}} \quad (\text{Eq. 4})$$

With SDME, the analyte transfers from the aqueous phase to the organic phase. By definition, the partition coefficient uses the activity of the analyte in the two phases. The activity is equal to the concentration of the analyte multiplied by the activity coefficient. Because low concentrations of analytes ($[\text{caffeine}] = 10^{-6} \text{ M}$) were used, the activity coefficients are assumed to be one, resulting in a practical form of the partition coefficient equation (Equation 5).³

$$K = \frac{[\text{analyte}]_{\text{organic}}}{[\text{analyte}]_{\text{aqueous}}} \quad (\text{Eq. 5})$$

Application to undergraduate laboratory.

Quantitative analysis using SDME with GC-MS has many potential applications to various analytes. The procedure can be easily adapted to SDME for use in the undergraduate analytical chemistry laboratory. The analytes are readily available in the average laboratory and samples are available within the detection limits and limits of quantitation of the procedure. The technique is not especially time-intensive and could be completed in a few laboratory periods, depending on the number of instruments available and the number of students in the course. However, the procedure requires manual operation in order to suspend and retract the microdrop of organic solvent in the aqueous solution. The technique has not been perfected with automated technology. The precision and sensitivity of the technique has improved with the study of various analytes and the optimization of analytes.¹¹⁻¹⁴ Problems can arise because it is relatively easy for the drop to dissolve in solution or dislodge from the tip of the solution.¹⁵ As of now, no educational undergraduate laboratory procedures have been published using static SDME and gas chromatography paired with mass spectrometry.

EXPERIMENTAL**Chemicals.**

All organic solvents were of HPLC grade from Fisher Scientific, including methanol, toluene and chloroform. Caffeine (99%) and N, N-diethyl-3-methylbenzamide (DEET, 97%) was purchased from Aldrich Chemical Company, Inc. All stock solutions were prepared by dissolving the standards in Nanopure Water.

Apparatus.

A Chaney adapter was attached to a 10- μ L syringe (Figure 4). The aqueous solution (4-mL) was held in an 8-mL amber glass vial with a rubber septum lid. A magnetic stir bar was placed in the bottom of the amber glass vial containing aqueous solution. The syringe was inserted into the

vial until the needle tip was submerged about one centimeter (Figure 5). The vial was held in place on a stirring plate by a clamp. During extraction, the syringe was held in place by a second clamp.

Extraction procedure.

The microsyringe was flushed with fresh organic solvent at the beginning of each day to ensure the removal of air from the syringe. The desired volume of organic solvent was withdrawn into the syringe to the preset total volume (see Appendix for Chaney adapter instructions). The needle was inserted into the amber glass vial containing the aqueous solution through the rubber septum. The needle tip was placed about one centimeter below the surface of the aqueous solution. The aqueous solution was stirred using an x shaped magnetic stir bar at 200 rotations per minute. The rate of stirring was maintained by the stir plate on which the amber glass vial was placed. The plunger was depressed to release the drop of appropriate volume (0.5 μL) onto the tip of the needle. The drop was suspended in the aqueous solution to allow for sufficient extraction (5 minutes). The drop was withdrawn into the microsyringe. The needle was removed from the rubber septum. The total microsyringe volume was injected into the GC-MS.

Sample Preparation

All caffeine and DEET samples were filtered by gravity filtration (Whatman No. 42 Ashless Filter Paper). If carbonated, the caffeine samples were degassed by sonification (Fisher Scientific Solid State/Ultrasonic FS-14) for 10 minutes. If necessary, samples were diluted with Nanopure water to avoid overloading the mass spectrometer. Samples included Diet Coke, Barq's Root Beer, XYIENCE XENERGY cran/razz energy drink, Twinings Lady Grey Tea, and Stash Vanilla Chai Decaf Tea.

GC-MS parameters.

An HP5890 Series II GC-MS was installed with a HP-1 column (internal diameter 0.2mm, film 0.33 μ m). A 2.5 minute solvent delay was used to prevent detection of the organic solvent. The injector and detector temperatures were 250°C and 280°C respectively. A splitless injection (1 minute purge delay) was used for all trials. The column head pressure was set at 22 psi. Oven temperatures varied for caffeine and DEET analysis. Caffeine was analyzed with GC oven temperatures of 150°C for 1 minute, 20°C per minute increase to 230°C for 2 minutes. DEET was analyzed with GC oven temperatures of 150°C for 5 minutes, 10°C per minute increase to 270°C for five minutes.

SIM m/z ratios.

Trials were completed using single-ion monitoring (SIM) mode on the mass spectrometer. The optimum mass/charge (m/z) ratios were determined individually based on the four characteristic peaks of caffeine and DEET. The characteristic peaks were tested with variations of ± 0.2 . The m/z ratios with the largest peak areas were chosen as the standard parameters for the subsequent trials. The characteristic peaks for caffeine were 194.1, 109.2, 82.1, and 52.9. The characteristic peaks for DEET were 190.1, 119.1, 91.1, and 65.1.

Integration parameters

All peak areas were determined using the integration software connected to the GC-MS. The initial threshold indicates the height required for a peak to be integrated. At lower concentrations, the initial threshold was optimized at 5.0. At higher concentrations, the initial threshold had the lowest relative standard deviation at 0.0. The peak width describes the minimum width of a peak at one half height required for integration. For all trials the initial peak width was kept at 0.010. The initial area reject is the minimum area below a peak required for integration. For all trials, the initial area reject was 0.0 because SIM mode was used

primarily, allowing for the assumption that any peak was the analyte if it was detected considering the other two integration parameters.

Flow rate

The flow rate was determined by injecting 1.0 μL of air into the column and measuring the retention time of air (m/z ratio = 28). The flow rate of the helium carrier gas was determined to be about 0.50 milliliters/minute when the column head pressure was kept at 22 psi.

Other applications

Ethanol in mouthwash

Single drop microextraction was used to analyze the amount of ethanol in mouthwash. This procedure was adapted from a similar experiment using small volumes (1 milliliter) to extract the ethanol from mouthwash using butanol, with propanol as an internal standard. In order to avoid dissolution of the solvent drop, headspace-single drop microextraction (HS-SDME) was used. The drop of butanol spiked with 12% (by volume) propanol was suspended in the vapor of the mouthwash. An amber glass vial with 4 milliliters of mouthwash was placed in a warm water bath (50-60°C) in order to establish equilibrium between the liquid and vapor phase of mouthwash. The solution was allowed to equilibrate, to ensure the vapor had formed in the headspace above the partially filled vial (Figure 15). After a five minute extraction with 1 μL of 12% propanol in butanol, the drop was injected into gas chromatograph with a thermal conductivity detector (GOW-MAC). The column was 20% Carbowax 20 μm on Chromosorb-P, 4ft by $\frac{1}{4}$ inch, 80/100 mesh). The parameters of the GOW-MAC run for ethanol analysis can be found in Table 6.

Phenol in Chloraseptic® spray

With SDME Chloraseptic® Sore Throat Spray is analyzed for the amount of phenol using a GOW-MAC (as seen in ethanol in mouthwash analysis). The parameters of the GOW-MAC run for

phenol analysis can be found in Table 7. One microliter of toluene was suspended in 4 mL of Chloraseptic® spray for 5 minutes while stirring at 200 rotations per minute. After the extraction, the toluene solution was injected into the GC-TCD (GOW-MAC). A calibration curve was constructed based on a series of diluted phenol solutions ([phenol] = 4.68 – 37.4 mg/mL.

RESULTS AND DISCUSSION

Caffeine.

For each trial a chromatogram and mass spectrum were recorded. The caffeine peak (retention time = 5.9 minutes) in the gas chromatogram (Figure 6) was identified by the four m/z ratios of the mass spectrum (Figure 7) in SIM mode. The smaller peak at 4.1 minutes was a result of impurities in the toluene drop. The percent relative standard deviation of the peak areas in the caffeine extracted into toluene calibration curve (Figure 8) ranged from 7 to 52%. The detection limit for this calibration curve was determined to be 1.2×10^{-2} mg/mL. The partition coefficient for caffeine extracted into toluene was 13 ± 5 . This value was calculated by dissolving caffeine directly into toluene in order to determine the actual amount of caffeine extracted into the organic drop during extraction. By comparing the concentrations of caffeine in water and in toluene, the partition coefficient was determined. The percent relative standard deviation of the peak areas for caffeine extracted into chloroform (Figure 9) ranged from 2 to 38%. The detection limit for this calibration curve was determined to be 2.6×10^{-3} mg/mL. The partition coefficient for caffeine extracted into chloroform was 250 ± 110 . Previous studies analyzed caffeine content in beverages with chloroform as the extracting solvent, using drop-to-drop solvent microextraction. This method had percent relative standard deviations between 3 and 7.3.⁹

Sample analysis

Several beverages (Diet Coke, Barq's Root Beer, Xenergy, Lady Grey Tea, and Decaf Chai Tea) were analyzed for their caffeine content. Both Diet Coke and Barq's Root Beer were close to the reported value (Table 1). The Xenergy energy drink reported value for proprietary blend included other energy supplements (taurine, glucoronolactone, guarana, panax ginseng, inositol, and L-carnitine) along with caffeine. According to one source, the amount of caffeine in the energy drink is 0.422 mg/mL.¹⁷ The reported caffeine concentrations for tea vary because reported tea values are not specific to the brand or type of tea. The caffeine content in brewed tea also varies depending on the brewing time, temperature, tea leaf composition and age, processing conditions and storage.¹⁸

DEET.

The DEET peak (retention time = 7.6 minutes) in the gas chromatogram (Figure 10) was identified by the four m/z ratios of the mass spectrum (Figure 11) in SIM mode. The percent relative standard deviation for the peak areas of DEET extracted into toluene (Figure 12) ranged from 6 to 24%. The detection limit for this calibration curve was determined to be 2.1×10^{-2} mg/mL. The partition coefficient for DEET extracted into toluene was 14 ± 4 . The percent relative standard deviation for the peak areas of DEET extracted into chloroform (Figure 13) were 2 to 64%. The detection limit for this calibration curve was determined to be a 6.6×10^{-3} mg/mL. Potential sample analysis would include OFF® Bug Spray, which contain 5 - 98.1% DEET by volume.¹⁹ At the lowest concentration the product DEET concentration could be detected using SDME. Surface water samples contain anywhere from 5×10^{-8} mg/mL to 1.1×10^{-6} mg/mL of DEET.²⁰ The detection limit of SDME does not allow for stream water sample analysis for DEET.

Optimization of method.

In order to perform the best extraction possible, it was necessary to optimize procedural parameters. The parameters included the extracting solvent, drop size, stirring parameters, and extraction time. The optimal parameters were determined using the lowest percent relative standard deviation between trials and the largest peak area.

Extracting solvent

Chloroform and toluene were used as the extracting solvents for the SDME of both caffeine and DEET from aqueous solutions (Table 2). Both solvents yielded stable drops on the tip of the syringe when suspended in aqueous solutions. The solvents were suitable for analysis in the GC-MS. The higher partition coefficient (250 vs. 13) and lower percent relative standard deviation indicate that chloroform is a better organic solvent for caffeine analysis. This agrees with the conclusions made in previous studies using SDME to analyze caffeine.⁹ At higher concentrations chloroform was a better organic solvent for DEET extraction than toluene as illustrated by the lower percent relative standard deviation.

Drop size

The drop of extracting solvent suspended in the aqueous solution was 0.5 μL throughout the trials. Trials with larger drops (1, 1.5, and 2 μL) resulted in the drop falling off the tip of the syringe. It was assumed that smaller drops of extracting solvent (less than 0.5 μL) would be less sensitive at lower concentrations.

Stirring parameters

The magnitude of the peak area was increased by stirring the aqueous solution during extraction (Table 3). The maximum stirring rate was 200 rotations per minute, which did not dissolve the drop during the extraction period. Faster rates caused the drop to fall off the tip of the syringe into the aqueous solution. The shape of the magnetic stir bar did not have a

significant effect on signal strength or precision (Table 4), although it was necessary for the stir bar to rotate in the bottom of the vial without hitting the sides of the vial. The x-shaped magnetic stir bar was chosen.

Extraction time

The extraction was optimized at 5 minutes (Table 5). Five minutes did not give the largest peak areas compared to seven and ten minute extractions; however the percent relative standard deviation was lowest for five minutes. Previous studies indicated that the dissolution of the organic drop over time result in a less precision.¹² The three and five minute extraction times had similar percent relative standard deviations, and the peak area for five minutes was nearly twice as large compared to three minutes.

Internal standards.

Internal standards were tested in an attempt to increase precision in the calibration curves and sample analysis. The use of *p*-xylene as an internal standard with DEET was ineffective (Figure 14). The toluene drop was spiked with 18.2 mg/mL *para*-xylene. In theory, there would be a linear relationship between the ratio of the peak areas of *p*-xylene to DEET and the concentration of DEET. As the concentration of DEET increases, the ratio of the peak areas of *p*-xylene to DEET should decrease, due to the constant concentration of the *p*-xylene in the drop of toluene. However, the percent relative standard deviations of the peak area ratio between *p*-xylene and DEET for the calibration curve are larger than those for the calibration curve without an internal standard (Figure 14).

Caffeine and DEET were not suitable internal standards for each other. The ratio between the two peak areas indicated inconsistencies in the composition of the toluene drop after extraction. The percent relative standard deviation of the ratio of the peak areas between

caffeine and DEET was 33% for a standard aqueous solution of caffeine and DEET. It would appear that DEET and caffeine compete to be extracted into the toluene drop, causing the imprecision in the ratio of DEET to caffeine.

Other applications

Ethanol in mouthwash

Single drop microextraction was adapted to analyze the amount of ethanol in mouthwash.

Based on a 12% ethanol standard, the Scope[®] mouthwash was found to have 30% by weight ethanol, compared to the 15% by weight reported value. It is possible that the concentration of ethanol in the headspace was higher than the aqueous volume.

Phenol in Chloraseptic[®] spray

The Chloraseptic[®] spray contained 11% phenol compared to the 1.4% reported value. A more extensive calibration curve may result in better accuracy in regards to phenol detection (Figure 16). One trial at each standard concentration was not suitable for a linear calibration curve ($R^2 = 0.9196$).

CONCLUSION

Chloroform was a better organic solvent than toluene when analyzing caffeine (detection limit = 1.1×10^{-3} mg/mL) and DEET (detection limit = 6.6×10^{-3} mg/mL). When analyzing caffeine and DEET, a good internal standard was not found.

Single drop microextraction (SDME) was a suitable procedure for an undergraduate laboratory. The procedure was easily learned and adapted to analyze caffeine in beverages, DEET in insect repellents, phenol in Chloraseptic[®] spray, ethanol in mouthwash including analysis on GC-MS and GOW-MAC. Potential analytes include caffeine, DEET, ethanol, and phenol. Instrumentation used includes GC-MS and GOW-MAC. The equipment and chemicals necessary are found in most undergraduate laboratories. The Chaney adapter was a useful

addition to the microsyringe, but was not necessary for all experiments. It was most beneficial if an intermediate volume was required. For example, if one microliter was withdrawn into the syringe, a 0.5 microliter drop was exposed to the aqueous solution. The small amounts of solvents necessary reduce the amount of organic waste.

This procedure is a valuable addition to any undergraduate laboratory because the variety of analytes and instrumentation gives students an opportunity to examine topics of interest. It is possible to compare preparatory procedures and instrumentation for the analysis of a particular analyte. For example, students could analyze caffeine concentration in beverages using SDME and GC-MS or HPLC. Additionally, the amount of ethanol in mouthwash could be determined using liquid-liquid extraction or SDME and analyzed with GOW-MAC. The procedures and instrumentation can be adapted to any class based on the laboratory skill level and understanding of chemistry.

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19. OFF Insect Repellents: What is DEET and why is it important?
<http://www.offprotects.com/deet/>
20. National Pesticide Information Center: DEET Technical Fact Sheet.
<http://npic.orst.edu/factsheets/DEETtech.pdf>

APPENDIX A - FIGURES AND DIAGRAMS

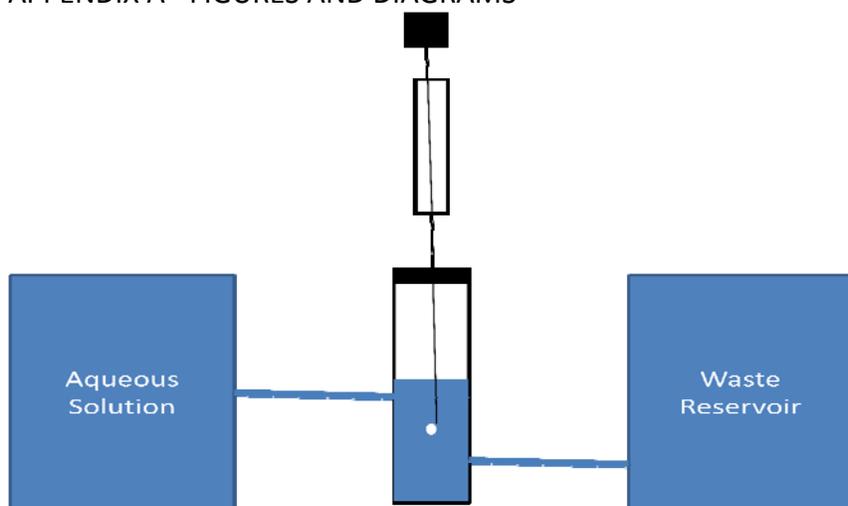


Figure 1. Cyclic flow SDME: aqueous solution flows around the organic drop in a circular pattern before flowing into the waste reservoir through a pump system.

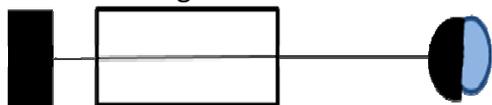


Figure 2. Bell device attached to tip of microsyringe. The filled bottom surface allows for better drop adherence but does not deliver a uniform drop volume and surface area.

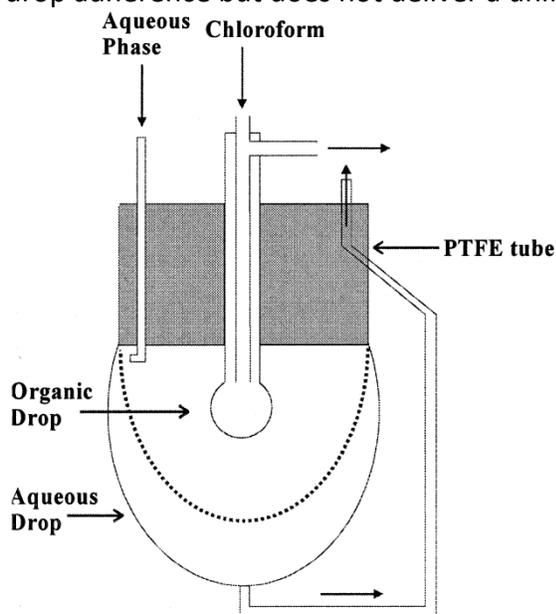


Figure 3. Drop-to-drop solvent microextraction. Organic drop suspended in aqueous drop on the tip of a microsyringe with fresh aqueous solution cycling around the organic drop.

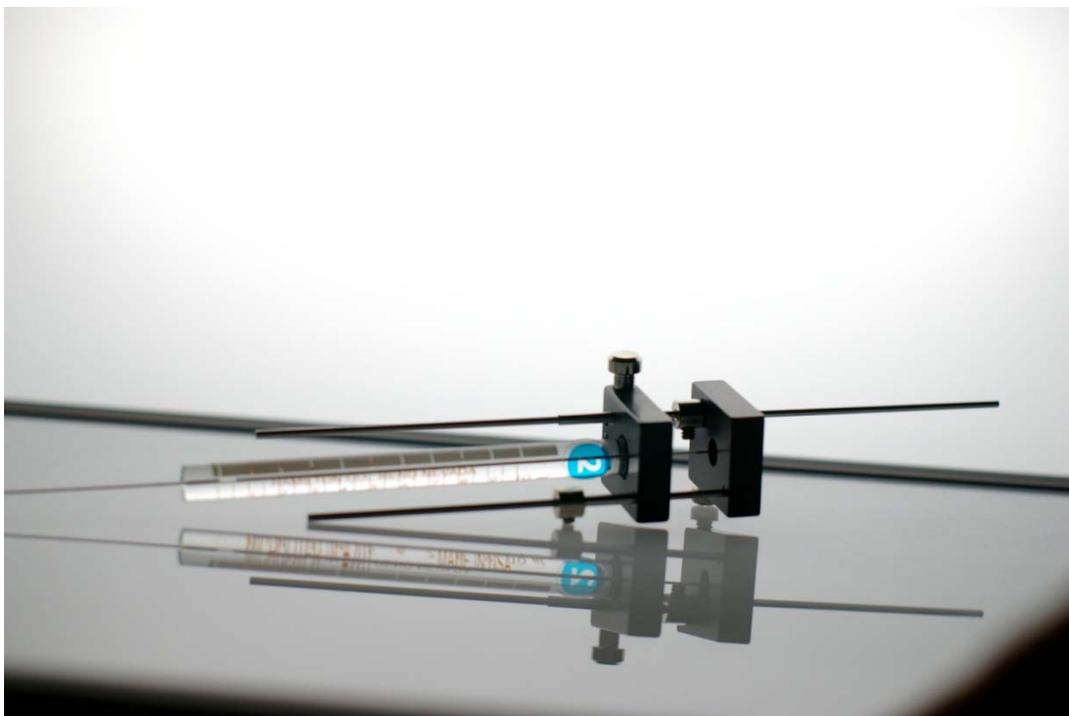


Figure 4. Chaney adaptor attached to 10 microliter syringe (Photo courtesy of Anjelika Gasilina).

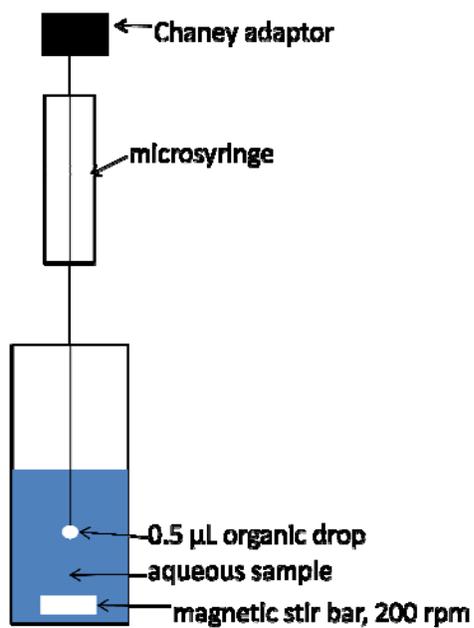


Figure 5. Standard experimental apparatus for all SDME trials.

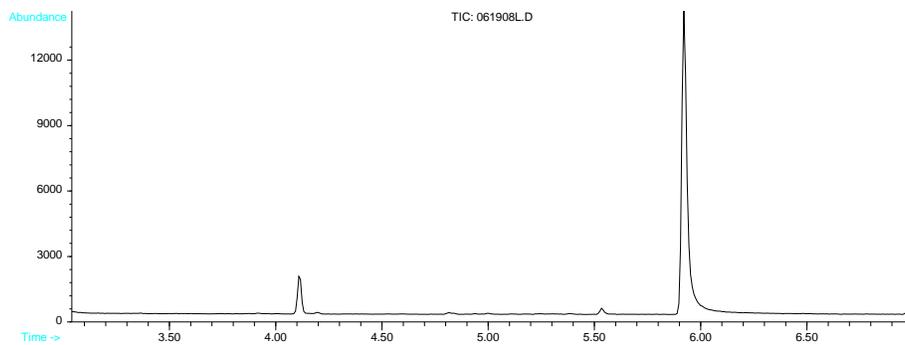


Figure 6. Aqueous caffeine (9.8×10^{-3} mg/mL) extracted into toluene gas chromatogram (SIM mode).

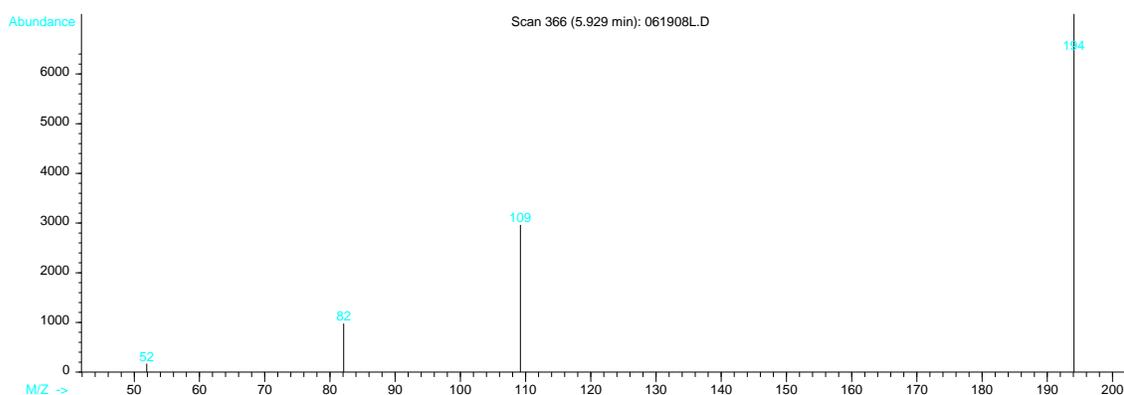


Figure 7. Aqueous caffeine (9.8×10^{-3} mg/mL) extracted into toluene mass spectrum.

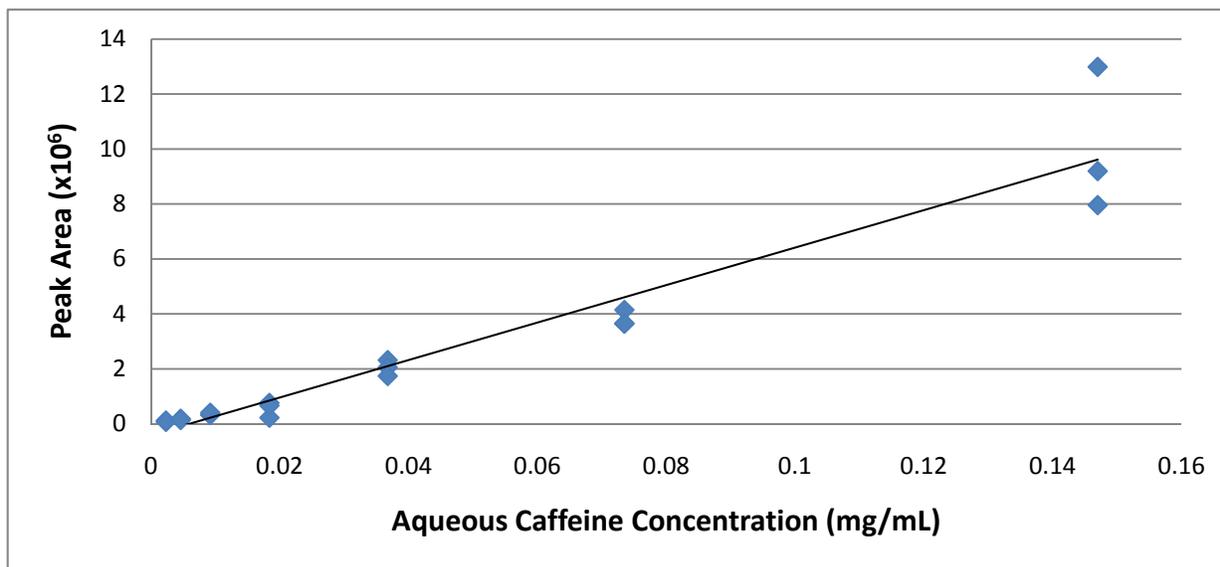


Figure 8. Calibration curve for aqueous caffeine extracted into toluene $y=6.8(\pm 0.4) \times 10^7 x - 4.1(\pm 2.8) \times 10^5$; detection limit = 1.2×10^{-2} mg/mL correlation coefficient = 0.930.

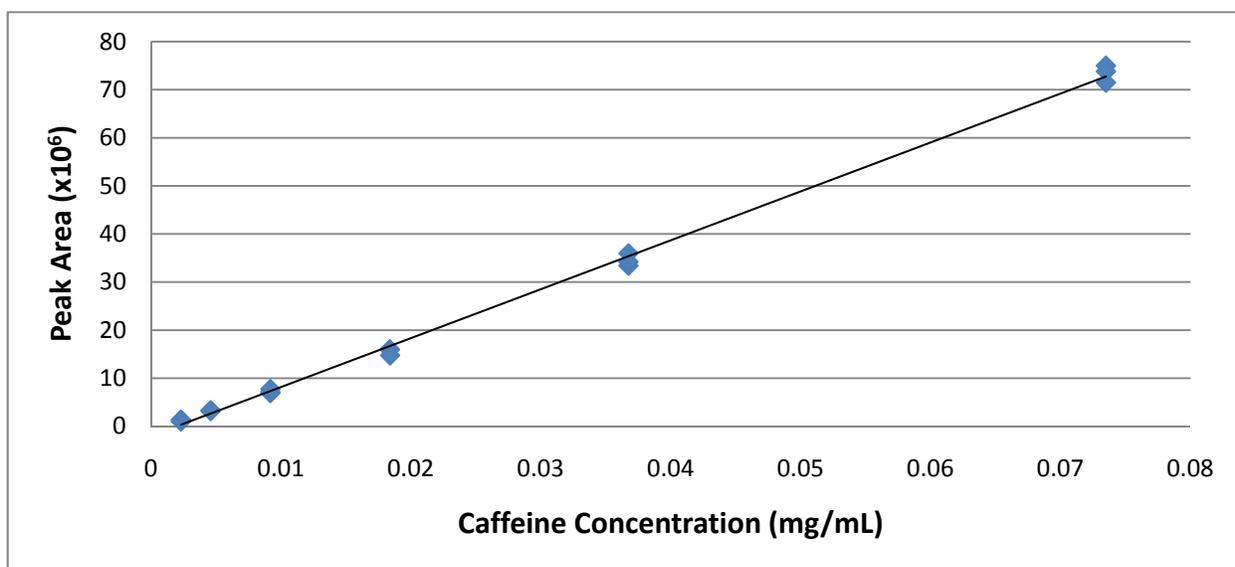


Figure 9. Calibration curve for aqueous caffeine extracted into chloroform $y=1.017(\pm 0.011)\times 10^9x- 2.0(\pm 0.4)\times 10^5$; detection limit = 1.1×10^{-3} mg/mL correlation coefficient = 0.998.

Table 1. Caffeine content of beverages analyzed using SDME (mg/mL).

	Diet Coke (mg/mL)	Barq's Root Beer (mg/mL)	Xenergy (mg/mL)	Lady Grey Tea (mg/mL)	Decaf Chai Tea (mg/mL)
Chloroform	0.092±0.011	0.054±0.011	0.292±0.011	0.201±0.011	0.017±0.011
Toluene	0.130±0.012	0.081±0.012	0.529±0.030	0.413±0.023	0.044±0.013
Reported⁵	0.13	0.064	2.8	0.19	0.0084

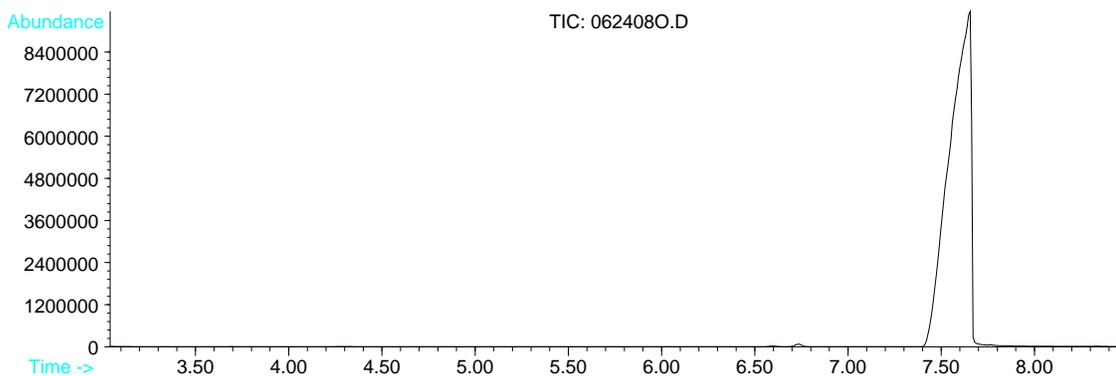


Figure 10. DEET (1.2×10^{-1} mg/mL) extracted into toluene gas chromatogram.

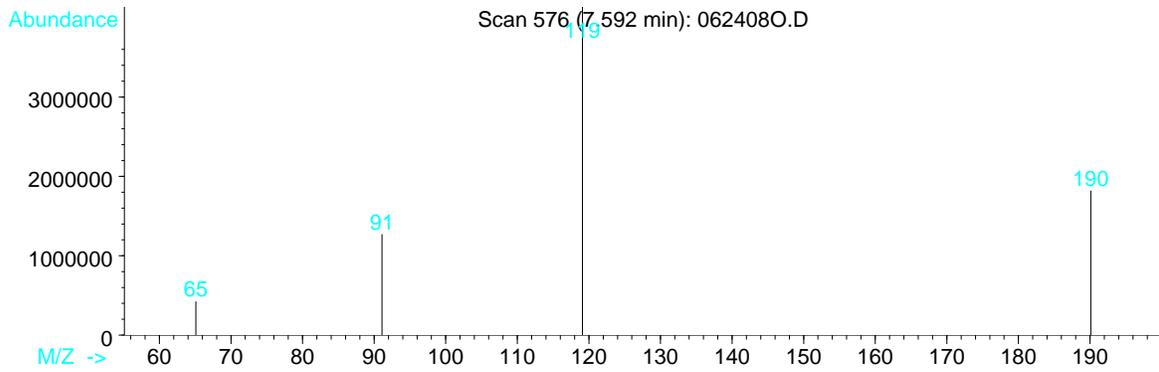


Figure 11. DEET (1.2×10^{-1} mg/mL) extracted into toluene mass spectrum.

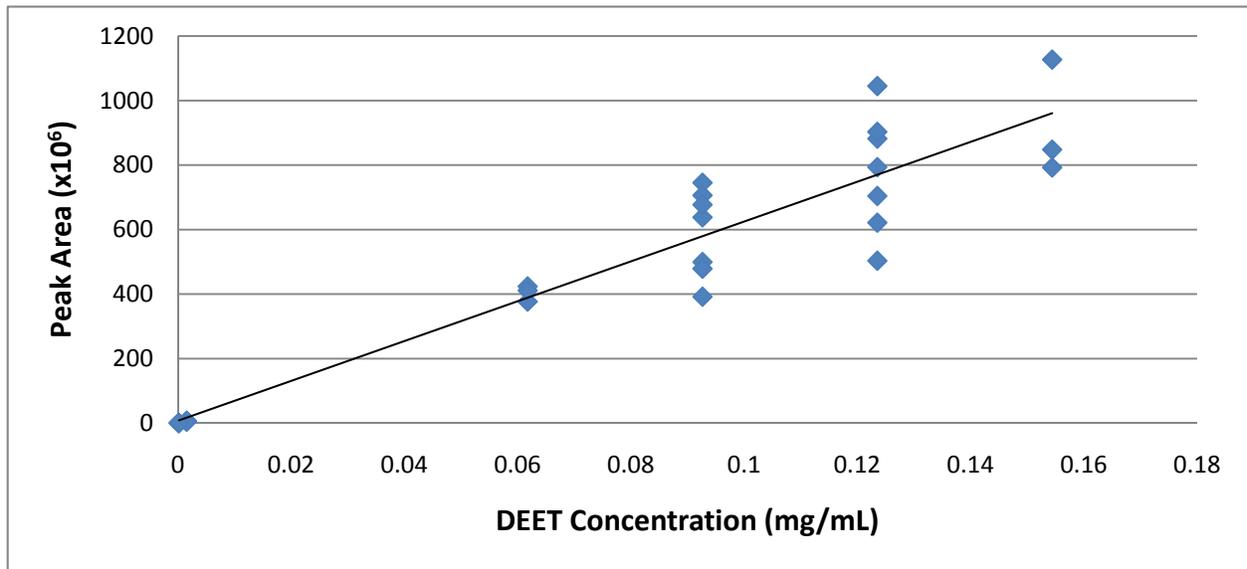


Figure 12. Calibration curve for DEET extracted into toluene $y=6.2(\pm 0.5) \times 10^9 x - 0.8(\pm 4) \times 10^6$; detection limit = 2.1×10^{-2} mg/mL correlation coefficient=0.883.

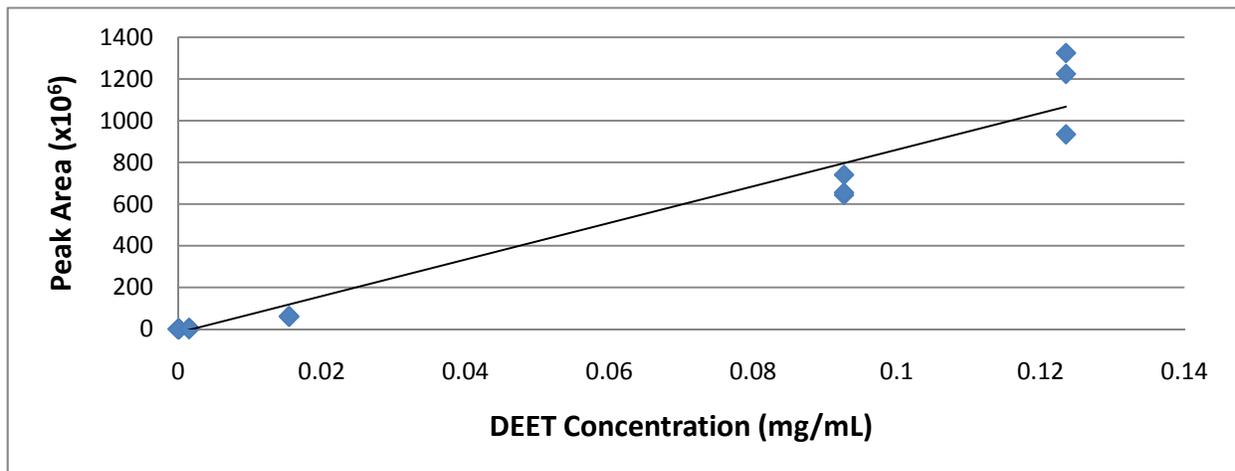


Figure 13. Calibration curve for DEET extracted into chloroform $y=8.8(\pm 0.4) \times 10^8 x - 1.7(\pm 1.9) \times 10^7$; detection limit = 6.6×10^{-3} mg/mL correlation coefficient = 0.960.

Table 2. Percent relative standard deviation of extracting solvents (n=3).

	Toluene	Chloroform
Caffeine % RSD	22	6.3
DEET % RSD	24	14

Table 3. Comparison of stirring rates (n=3) for the extraction of caffeine (9.8×10^{-3} mg/mL) into toluene using SDME.

	0 rpm	200 rpm
Average peak area	173000	240000
Standard deviation	6000	50000
% RSD	4	20

Table 4. Comparison of stir bar shapes (n=3) for the extraction of caffeine (9.8×10^{-3} mg/mL) into toluene using SDME.

	x stir bar	Cylinder
Average peak area	240000	230000
Standard deviation	50000	50000
% RSD	20	20

Table 5. Comparison of extraction times (n=3) for the extraction of DEET (0.062 mg/mL) into toluene using SDME.

	3 minutes	5 minutes	7 minutes	10 minutes
Average peak area	2.18×10^8	4.0×10^8	4.9×10^8	8.3×10^8
Standard deviation	1.8×10^7	2.4×10^7	6×10^7	2.2×10^8
% RSD	8.4	6.0	10	26

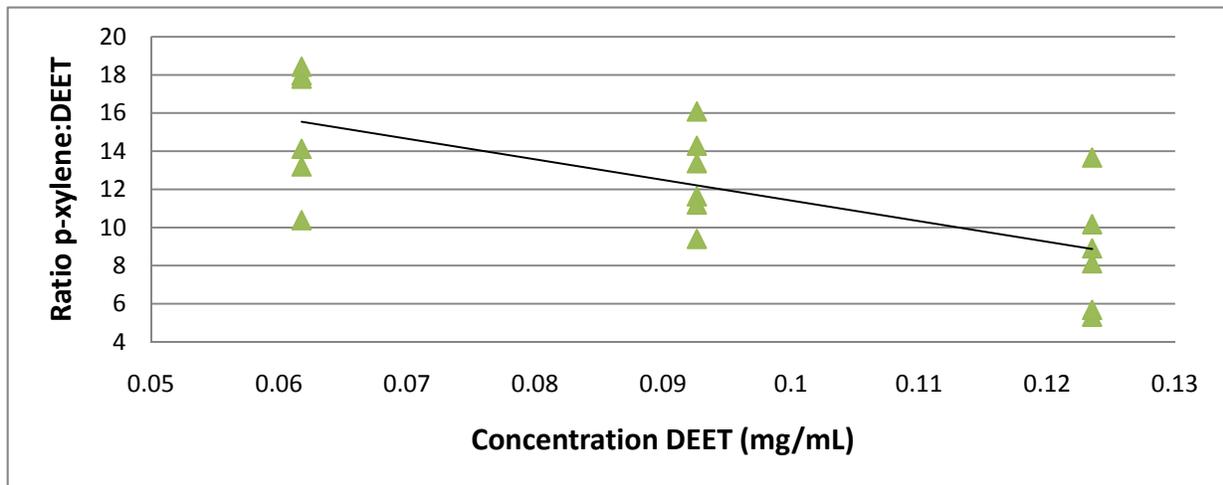


Figure 14. Peak area ratio of para-xylene to DEET versus the concentration of DEET (mg/L); $y = -108 (\pm 27) x + 22.2 (\pm 2.6)$; correlation coefficient = 0.504.

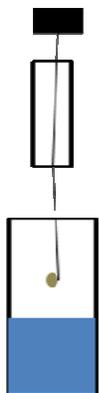


Figure 15. Headspace single drop microextraction apparatus.

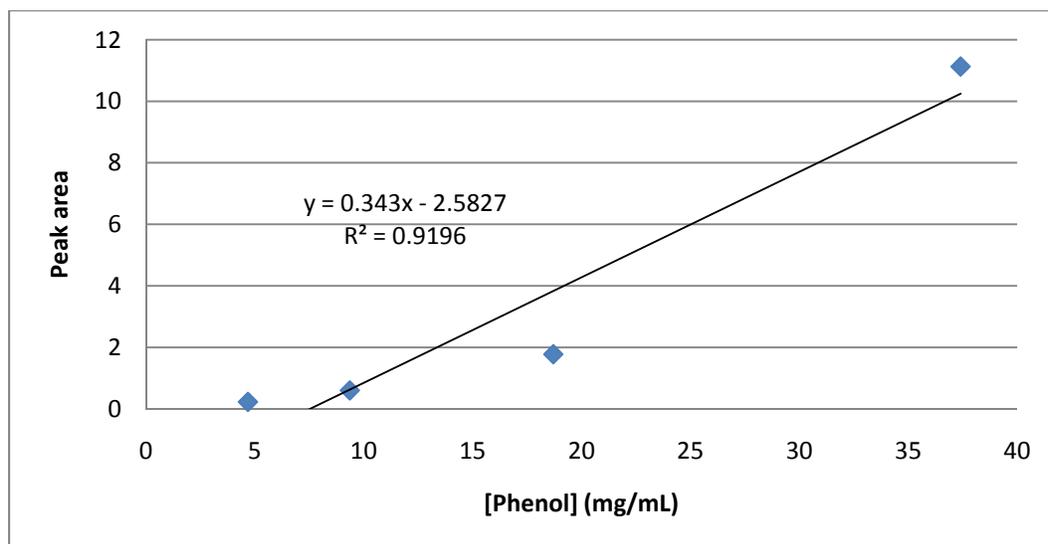


Figure 16. Standard phenol solution calibration curve; $y=0.34 \pm 0.07 x - 2.6 \pm 1.5$; correlation coefficient = 0.9196.

Table 6. Experimental parameters for the analysis of ethanol in mouth wash with GC-TCD.

Column temperature (°C)	98
Detector temperature (°C)	201
Injector temperature (°C)	182

Table 7. Experimental parameters for the analysis of phenol in Chloraseptic® spray with GC-TCD.

Column temperature (°C)	202
Detector temperature (°C)	217
Injector temperature (°C)	213

APPENDIX B - STUDENT WORK

Experimental

Students in Chemistry 382 at Wittenberg University during the fall of 2009 used single drop microextraction during an undergraduate laboratory. Due to the length of each trial, it took approximately two hours for each lab group to complete 12 trials (5 standards and 1 sample, 2 trials each). The sample dilution calculations (Table 9) were available to the students, reducing the time required in the preparatory laboratory which allowed for more trials. The written procedure (see Appendix D) was clearly understood, although use of the Chaney adapter was better communicated verbally (see Appendix E).

Results

As a collective group, the students were able to construct a collaborative calibration curve with good linearity in SIM mode (Figure 16). The percent relative standard deviation between the trials was comparable to other trials, regardless of the group performing the trial (Table 8). The students were able to suspend 0.5 microliter drops of toluene into aqueous solutions of caffeine with success and precision.

Future Work

In the future, students would benefit from practicing with the Chaney adapter using water and toluene. A run in SCAN mode at the beginning of the trials is a good illustration of how the instrument works and to explain the choice of mass-charge ratios.

APPENDIX C – STUDENT WORK – FIGURES AND DIAGRAMS

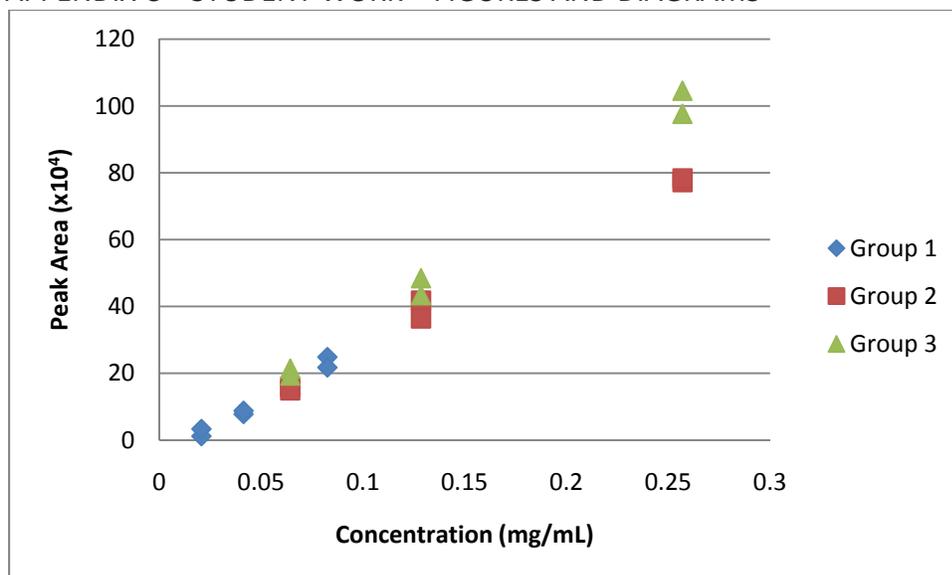


Figure 16. Calibration curve for caffeine extracted into toluene from CHEM 382 class (Fall 2009). $y=34.2(\pm 1.1) \times 10^5 x - 5.0(\pm 2.0) \times 10^4$; detection limit = 18.6×10^{-3} mg/mL; correlation coefficient = 0.961.

Table 8. Statistical calculations of data collected from caffeine standards of varying concentrations in CHEM 382 (Fall 2009).

[caffeine](mg/mL)	Average peak area	Standard deviation	%RSD
0.0206	2.8×10^4	1.3×10^4	48
0.0321	1.1×10^5	3×10^4	30
0.0413	8.3×10^4	7×10^3	8.5
0.06425	1.7×10^5	4×10^4	23
0.0825	2.35×10^5	1.6×10^4	6.7
0.1285	3.9×10^5	6×10^4	16
0.165	4×10^5	9×10^4	22
0.257	8.9×10^5	1.4×10^5	15
0.517	1.7×10^6	2.4×10^5	14

Table 9. Serial dilution information provided to CHEM 382 students

[caffeine] _i (mg/mL)	Volume _i (mL)	[caffeine] _f (mg/mL)	Volume _f (mL)
0.5140	25	0.2570	50
0.2570	25	0.1285	50
0.1285	25	0.0643	50

APPENDIX D - LABORATORY PROCEDURE FOR STUDENTS

Calibration Curve Solution Preparation. Prepare a stock solution of caffeine dissolved in water (0.514 mg/mL). Use the stock solution to make solutions for a calibration curve by serial dilution. Solution was prepared by Betty Cheney on September 25, 2009.

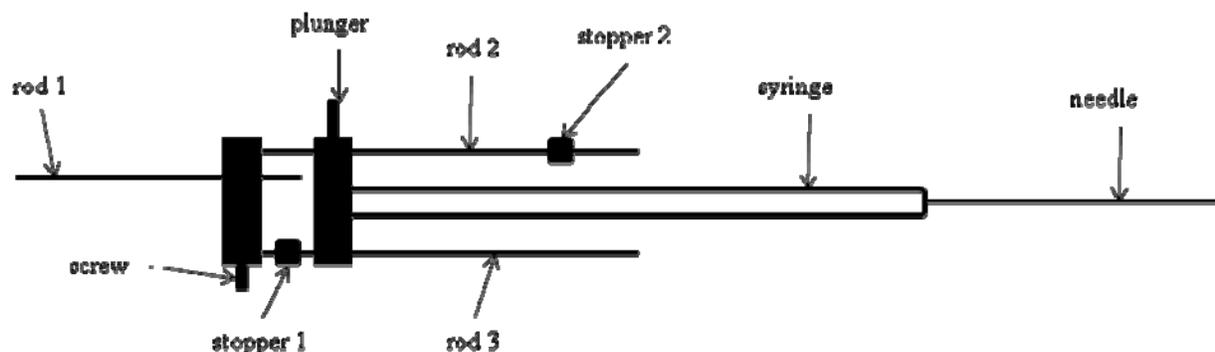
Caffeine Sample Preparation. Prepare carbonated caffeine samples (soda, energy drinks) by degassing (place in sonicator for ten minutes). Non-carbonated caffeine samples (coffee, tea) do not need to be degassed. All samples need to be filtered.

Single Drop Microextraction Procedure. Fill a small glass vial (about 7 mL) with aqueous caffeine solution (4 mL) and a small stir bar. Cap the vial with a rubber septum and lid. Place the vial on a stir plate set to 300 rotations per minute and secure using clamps. Attach a Chaney adapter to a 10-microliter microsyringe. Fill the microsyringe with 0.5 microliters of toluene, checking for air bubbles. Insert the syringe needle into the vial through the septum. Suspend a 0.5 microliter drop into the solution. After five minutes, the drop is withdrawn into the syringe then injected into the gas chromatograph/mass spectrometer.

Gas Chromatograph/Mass Spectrometer Parameters. A 2.5 minute solvent delay is used to prevent the detection of the toluene solvent. The injector and detector are set to 250°C and 280°C respectively. A splitless injection (1 minute purge delay) is used. The column head pressure is set to 20 psi. The gas chromatograph oven temperature is set at 150°C for the first minute, 20°C/minute to 230°C for two minutes. Single ion monitoring mode is used at mass to charge (M/Z) ratios of 194.1, 109.2, 82.1, and 51.9.

Data Analysis. Each chromatogram is analyzed using computer software to integrate the area under each peak. By determining a caffeine calibration curve, it is possible to calculate the amount of caffeine in each solution.

APPENDIX E - CHANEY ADAPTER INSTRUCTIONS



Setting the volumes for the Chaney adapter

1. With the syringe needle in the sample, withdraw an excess amount of organic solvent into the syringe to wet the needle.
2. Set rod 1 using the screw on the upper plate of the adapter. The rod should be at the height to deliver the intermediate volume. The bottom of the rod will be stopped by the plunger, when the plunger is depressed
3. Set stopper 2 on rod 2 at the desired maximum volume.
4. Stopper 1 is set during each trial. It provides a stable way to deliver the same volume as set by rod 1 and the plunger.

Procedure during each trial

1. Fill the syringe to the desired maximum volume using stopper 2 on rod 2.
2. Insert the needle into the vial of aqueous solution through the rubber septum.
3. Depress the plunger on the Chaney adapter and allow the organic solvent drop to remain on the tip of the syringe.
4. While still holding the plunger, tighten stopper 1 on rod 3 to stabilize the volume of the drop during extraction.
5. Release the plunger once stopper 1 is tightened on rod 3 and extract for the desired amount of time.
6. After the desired extraction time, withdraw the drop back into the syringe, allowing stopper 2 to signal the complete withdraw of the solvent drop.
7. Inject the sample into the gas chromatograph/mass spectrometer, loosening stopper 1 in order to release the full volume of solvent into the column.
8. Rinse the syringe with solvent and repeat.