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Laboratory Experiment

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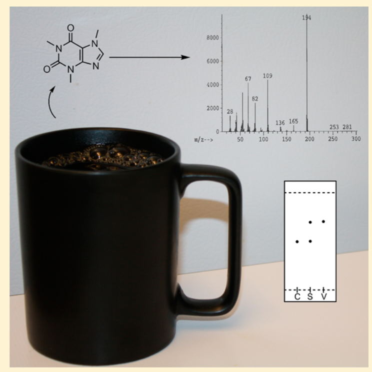
Separation of Caffeine from Beverages and Analysis Using Thin-Layer Chromatography and Gas Chromatography–Mass Spectrometry

Janelle L. Torres y Torres,* Shauna L. Hiley, Steven P. Lorimor, Jonathan S. Rhoad, Benjamin D. Caldwell, Gerald L. Zweerink, and Michael Ducey

Chemistry Department, Missouri Western State University, St. Joseph, Missouri 64507, United States

Ⓢ *Supporting Information*

ABSTRACT: The Characterization and Analysis of a Product (CAP) project is used to introduce first-semester general chemistry students to chemical instrumentation through the analysis of caffeine-containing beverage products. Some examples of these products have included coffee, tea, and energy drinks. Students perform at least three instrumental experiments as a part of this five-part project to analyze different components of the beverage and its packaging. In this discussion, the first of these experiments is presented. Caffeine and other components, such as flavorings, are extracted from the product using dichloromethane. The extract is analyzed using thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC–MS) to identify caffeine and other trace components. Students also calculate the percent abundance of the ^{35}Cl and ^{37}Cl isotopes from the dichloromethane mass spectrum. These exercises demonstrate several basic concepts introduced in the first-semester course, and are easily adaptable to using in several courses in the undergraduate curriculum.



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Integration of instrumentation throughout the entire four years of the chemistry curriculum is a means of preparing students to become confident users of instrumental methods, with the goal of translating these skills to those needed on the job or in graduate school. Early integration of chemical instrumentation has been a cornerstone of the Missouri Western State University (MWSU) chemistry curriculum for decades. The introduction of chemical instrumentation in the General Chemistry I course, since 1998, has allowed us to draw threads to both real-world analyses of products and a view of modern analysis using instrumentation students would actually use in local industry. We have developed the Characterization and Analysis of a Product (CAP) series, first described by Ducey and Caldwell,[1] that uses five experiments spread throughout the General Chemistry I course ("CAP 1" through "CAP 5") to guide students through the extended analysis of a caffeine-containing product, typically beverages such as coffees, teas, soft drinks, or energy drinks. This series is similar in approach to a recently developed series of experiments that utilizes a variety of instrumental and wet techniques to explore the composition of a tomato.[2] One advantage of the CAP series is its ability to be adapted to this variety of beverage products and likely others. The CAP series also introduces first-year students to instrumental methods that students might not be exposed to until much later in many chemistry program curricula. Three of the experiments in this sequence give these students exposure to the instrumental methods of gas chromatography–mass spectrometry (GC–MS, "CAP 2"), Fourier transform infrared spectrophotometry (FTIR, "CAP 3"), and atomic absorption spectroscopy (AAS, "CAP 4"). In "CAP 5" students design and carry out an experiment to answer a testable question they propose about their beverage, possibly utilizing instrumentation as well.[1]

The experimental sequence begins with an examination of the packaging and contents of the beverage container ("CAP 1") in which students write qualitative and quantitative observations and testable questions about the product and its packaging. This exercise is completed during the first week of lab and gives students an introduction to basic vocabulary and practice with the scientific method. Students use the same brand of beverage for all experiments in the sequence. Groups of three to four students are intended to stay together for the entire sequence of experiments.

Here we describe the "CAP 2" segment of this series, in which students extract caffeine from a beverage and characterize the extract using TLC and GC–MS. GC–MS is one of the most versatile tandem methods for quantitative and qualitative identification of sample components. Its use in the general chemistry laboratory curriculum has become more common in recent years, demonstrating the suitability of incorporating such instrumentation into an introductory course.[3−9] This experiment has been conducted with over 3500 on-campus and dual

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credit General Chemistry I students at MWSU. The detection of caffeine in beverages using high-performance liquid chromatography (HPLC), including coffee,[10,11] soft drinks,[11] and energy drinks;[12] nuclear magnetic resonance (NMR) of energy drinks;[13] and UV−visible spectrophotometry (UV−vis) of coffee extracts[14] has been reported for use in the instructional laboratory. Only two of these experiments[10,14] are targeted for the general chemistry laboratory. The ultimate goal of the "CAP 2" experiment is to successfully identify caffeine in the extract while potentially identifying other substances, such as those possibly added as flavoring agents, in the drink. The general chemistry concepts that are reinforced in this experiment include the theory of polar/nonpolar interaction through extraction, GC, and TLC. It also includes atomic structure and isotopes, which are addressed through the analysis of chlorine peaks in mass spectra. The experiment fits into two 3 h laboratory periods that can be placed virtually anywhere during the semester. It is most easily incorporated following the introduction of atomic mass calculations and the introduction of intermolecular forces. If used very early in the semester, it can give students a first look at intermolecular forces by introducing the "like dissolves like" principle. Combination of TLC with GC−MS also provides the opportunity to translate the TLC theory in steps to that of packed column GC and capillary GC in the prelaboratory demonstration.

■ EQUIPMENT AND MATERIALS

Extraction of Caffeine from Beverage (CAP 2 Part 1, First Week of Experiment)

Beverage samples are prepared by either degassing (with agitation) of carbonated drinks or by brewing coffee or tea according to package directions. The extraction protocol is modified from two previously published procedures.[15,16] Two grams of reagent grade anhydrous sodium carbonate is added to a 100 mL beverage sample in a 500 mL Erlenmeyer flask, which is covered and swirled until the sodium carbonate is dissolved. The sample is poured into a 250 mL separatory funnel in the fume hood and extracted by gently swirling with 25 mL of HPLC grade dichloromethane. The extract is collected in a preweighed 50- or 100 mL beaker and evaporated to near dryness on a hot plate. The remaining solvent is allowed to evaporate in the fume hood. The mass of the extract is then recorded and the beaker is covered and stored in the lab drawer until the following week.

Thin-Layer Chromatography (TLC) and Gas Chromatography−Mass Spectrometry (GC−MS, CAP 2 Part 2, Second Week of Experiment)

Students reweigh the beaker and add approximately 3 mL of dichloromethane using a glass pipet, and partition the extract into two portions: one for GC−MS analysis, and a second for TLC analysis. Student groups alternate between the GC−MS and TLC experiments.

Micropipettes, prepared from open-ended melting point capillary tubes, are used for spotting sample and caffeine standard (0.5 mg USP grade caffeine/mL in dichloromethane) onto a 1 in. × 2 and 1/3 in. polyester-backed silica gel TLC plate with fluorescent indicator. Plates are developed using 3:1 methanol/ethyl acetate (HPLC grade solvents)[16] in a 100 mL beaker covered with aluminum foil, using a cut piece of filter paper as a wick. Spots are visualized with a UV lamp. The distance to the center of each spot (d_{spot}) and the solvent front distance (d_{solv}) from the spot line are measured with a ruler and the R_f (retention factor) for each spot is calculated from $R_f = d_{spot}/d_{solv}$.

Approximately 1.5 mL of the redissolved caffeine extract is added to a GC autosampler vial enclosed by crimping an aluminum cap with a rubber septum over the vial. A Hewlett-Packard (HP, Agilent Technologies, Santa Clara, CA) 5890 II plus GC is used to separate a 1.0-μL sample of the extract over a Restek (Bellefonte, PA) 30 m RTX-5 MS 0.25 mm i.d. capillary column with a 0.25 μm i.d. film thickness using helium as a carrier gas. Sample components are detected with an HP 5972 MS (Agilent Technologies, Santa Clara, CA), accompanied by an HP Chemstation instrument control and data collection software package.

Specific conditions are included in the Supporting Information. The GC−MS method is only just over 12 min long, ensuring all eight groups in one section can complete the experiment during the 3 h lab period with sufficient time to answer end-of-lab questions. For all segments of the CAP 2 protocols and extensive notes for the instructor, please see the Supporting Information provided.

■ HAZARDS

Hot plates are used in the fume hood for the evaporation of the dichloromethane and represent a burn hazard. Ethyl acetate and methanol, used in the TLC experiment, are toxic and flammable and are also used in the fume hood. Dichloromethane is a suspected carcinogen and is used in the fume hood. Typical precautions are taken with separatory funnels. Students are taught the technique for gently swirling the extraction mixture and periodically releasing the pressure. The TLC micropipettes are prepared by instructors using glass capillary tubes and a Bunsen burner and provided to the students. HPLC grade methanol and dichloromethane used for GC−MS needle washes are used in the appropriate vials provided for the instrument. The GC−MS uses an autosampler, preventing any exposure of the student to the heated injection port of the GC. Students use small volumes in each part of the experiment. Caffeine is an irritant. Gloves are recommended when handling caffeine-containing extracts and solvents. Goggles are worn during all laboratory activities. Waste organic solvents are collected in properly labeled waste containers in the fume hood and are disposed of according to MWSU waste disposal guidelines.

■ RESULTS AND DISCUSSION

The caffeine extract (usually between about 1 and 20 mg) from tea and energy drinks is typically a white solid, but can be discolored to a light yellow to beige color. Students must take great care to not contaminate the extract with the aqueous layer. Suggestions are located in the Supporting Information. The TLC method uses commonly available supplies and laboratory glassware, facilitating its use in the general chemistry laboratory, such as 100 mL beakers, filter paper, and aluminum foil to cover the beaker (the "TLC chamber"). The TLC developing solvent efficiently separates caffeine (R_f = 0.50−0.55) from vanillin (R_f = 0.70−0.75).

For the GC−MS experiment, students record information for each compound identified to an 80% or better match to the spectral library, including the compound name, scan number, retention time, the library reference spectrum number, and the percent identity or "quality" of the match. They also print a

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mass spectrum for the solvent (dichloromethane), measure the height of the ^{35}Cl and ^{37}Cl peaks with a ruler, and calculate the percent abundance of each of these isotopes from this data. Pfennig and Schafer[5] also report an alternate method for calculation of the percent abundance of chlorine by GC−MS. Our method is able to detect caffeine in the coffee, as well as substances likely added as flavoring agents to the coffee, such as vanillin, by the comparison of the sample spectrum to the mass spectral library.

The TLC experiment gives students a preliminary indication of the presence of caffeine in the extract, while the GC−MS experiment is much more sensitive and gives a more conclusive confirmation of the presence of caffeine (95%+ match between sample MS and library MS). While this lab exercise was prepared for General Chemistry I, this experiment would certainly lend itself toward other courses with modification. The extraction, weighing of the caffeine extract, and TLC analysis portions of the experiment are straightforward enough to perform with most introductory or liberal arts chemistry courses that introduce intermolecular forces and polarity. This would typically require two lab periods. It also offers an analysis of a real-world product to pique the interest of students. Alternatively, this experiment could be easily adapted for an upper-level analytical course by having students develop the instrumental protocol for the GC−MS analysis, rather than use a program already prepared for the student, as we do in the General Chemistry I course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

A student handout for CAP 2 Part 1 and CAP 2 Part 2, extensive instructor notes, suggestions for grading, end-of-lab questions, and the key to the end-of-lab questions. This material is available via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jtorresytorres@missouriwestern.edu.

Author Contributions

S.L.H., G.L.Z., and B.D.C. developed the initial instrumental protocol and early modifications of the experiment. J.L.T.T. and J.S.R. developed the TLC analysis method, and J.S.R. created the abstract photo. M.D. and G.L.Z. designed the original CAP concept, and S.P.L. developed recent modifications to the GC−MS instrumental analysis protocol and made significant edits to the manuscript. S.L.H. and J.L.T.T. wrote the majority of the manuscript.

Notes

The authors declare no competing financial interest.

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