Teacher Notes for "How Much Caffeine Is in My Soft Drink?"
Determination of Caffeine Content in Soft Drinks Using GC-MS

Documents Included with this lesson:

1. "Caffeine Lab Teacher Notes.pdf" – teacher's notes about the experiment (this document)
2. "Caffeine Lab Protocol + Prelab.pdf" – student lab protocol for experiment with directions; also contains pre-lab assignment/questions
3. "Caffeine Lab Answers.pdf" – sample answers to (1) pre-lab assignment questions and (2) lab report calculations and analysis questions.
4. "caffeine_content_drinks.pdf" – chart with published caffeine content values in various soft drinks and energy drinks.
5. "Caffeine GC-MS.ppt" – PowerPoint about GC-MS; also explains the experimental methods and rationale for separation in this experiment.

Student Level:
All levels of high school chemistry, mathematical knowledge of ratios and unit conversions would be helpful when comparing concentration of normal caffeine with deuterated caffeine to calculate content of normal caffeine in the soft drink.

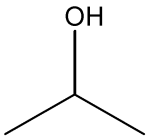
Experiment Notes and Pitfalls:

- Make sure the soft drinks are flat, the flatter the better. It is OK if the soft drinks are opened a day or two prior to the experiment and left on the counter. In a freshly opened can, the soft drink foam occupy volume, cause the experimental yield to be lower than expected.
- You pipet 100 μL of deuterated caffeine to each group. Since this is in limited quantity, you will want to control this.
- If you want to use a percentage other than 70% rubbing alcohol, you may calculate the volume of isopropanol. The relationship is inversely proportional. Divide 350 μL by the percentage expressed as a decimal, e.g. for 70% would be 350 μL / 0.70 = 500 μL of 70% rubbing alcohol needed. Round up; extra isopropanol is OK.

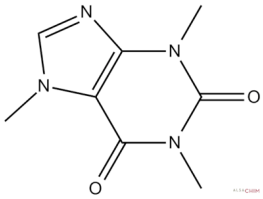
Content-Related Background Notes:
Density of 2-propanol: 0.785 g/mL at 25°C
Density of water: 1.000 g/mL at 25°C

Solubility of caffeine in 2-propanol: ??? at 25°C (but increased compared to water when sodium carbonate is introduced)
Solubility of caffeine in water: 2.17 g/100mL at 25°C

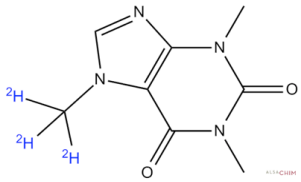
Structural formulas of some compounds used in this experiment:



← 2-propanol (isopropanol)
< http://wtt-lite.nist.gov/cgi-bin/openindex.cgi?cid=67630>



← caffeine
< http://www.alsachim.com/product-C505-drugs.metabolites-Caffeine-D3.html>



← deuterated caffeine (caffeine-d3)
< http://www.alsachim.com/product-C505-drugs.metabolites-Caffeine-D3.html>

Materials:

Apparatus (needed for each lab group)

- 25-mL beakers (2 per group / other size beakers OK)
- 1000 μL automatic pipets with disposable tips (can be shared between groups)
- Small test tube with stopper (should hold around 5 mL)
- Glass stirring rod
- Electronic balance and weighing paper
- Spatula
- GC-MS Autosampler 1.5-mL microvial***
- 100 μL automatic pipet with disposable tips (1 for instructor only)

Reagents (assuming 8 groups in the class)

- Soft drinks / energy drinks (exactly 1.000 mL each or 1/3 fl. oz.) – each group selects one to bring in
- 70% isopropyl alcohol or 2-propanol, a.k.a. rubbing alcohol easily found at drug stores, supermarkets, etc. (~20 mL per class)
- Anhydrous sodium carbonate (~1.5 g per class)
- Sodium sulfate (~1.0 g per class)
- deuterated caffeine, d_3-caffeine (1 mg/mL concentration, 1 mL volume)***
  ***Obtained for FREE from U.C. Berkeley Mass Spectrometry Facility
  E-mail Dr. Ulla Andersen (norklit@berkeley.edu) for information/to arrange.
  Contact 1 month prior just to be safe.

Outline:

Teacher Preparation of Materials (Approximate prep time: 15 minutes)

Do Step 1 one month in advance. The rest can be done 1 or 2 days prior to the lab.

1. ***E-mail Dr. Ulla Andersen (norklit@berkeley.edu) at U.C. Berkeley Mass Spectrometry Facility to arrange shipment of GC-MS Autosampler 1.5-mL microvials (1 per lab group) and deuterated caffeine. **Please contact 1 month prior to the lab just to be safe.**
2. Make sure you have apparatus materials (see previous page for list) needed for the experiment and the following reagents in your stockroom: anhydrous sodium carbonate (~1.5 g per class) and sodium sulfate (~1.0 g per class).
3. Obtain 70% isopropyl alcohol or 2-propanol (commonly known as rubbing alcohol), easily found at drug stores, supermarkets. Each class will need ~20 mL.
4. Have students sign up and bring soft drinks for analysis.

Background and Experiment (1 class period or about 45-50 minutes)

1. Explain to your students:
   - the relevance of this experiment in determining the caffeine content in soft drinks they are likely to consume
   - density (Isopropanol is less dense than water, so isopropanol floats on water.) **Optional Demonstration:** If you want to illustrate this, you may want to demo or have each lab group put about 10 mL of soft drink in a test tube with 5 mL of rubbing alcohol. Cap and invert the test tube twice (just as students will do during the experiment), venting as needed to relieve pressure. Have students observe what happens.
   - solubility (Caffeine is more soluble in isopropanol than water. Sodium carbonate makes sugars and other compounds more soluble in water by making the pH more basic and attracting the water molecules more tightly to itself.)
   - molecular polarity (Caffeine is slightly polar, allowing it to dissolve more readily in isopropanol than water when the ionic compound sodium carbonate is added. Sodium carbonate is attracted to the water more, so the caffeine goes along with the isopropanol.)
   - what deuterated caffeine is (Normal caffeine has a molecular weight of 194 and deuterated caffeine has a molecular weight of 197. Deuterated caffeine has 3 deuterium atoms. Deuterium is a hydrogen atom with an extra neutron. The mass difference in deuterated caffeine allows us to use a similar molecule with similar structure for comparison.)
   - Explain GC-MS. **Optional:** Use attached Powerpoint titled "Caffeine GC-MS.ppt". It contains background information about GC-MS and goes over the procedures for the experiment.
2. Students carry out the experiment in their student lab groups or pairs. **Teacher** pipets 100 μL of 1 mg/mL deuterated caffeine to each group, since this is substance is in limited quantity. The total procedure should take about 25 minutes.

- Make sure each group labels their sealed GC-MS Autosampler 1.5-mL microvial with class period and sample number. Number samples consecutively starting from #1.

3. Pack your caffeine samples in the same manner as you received the empty microvials. Mail (any except USPS) or self-deliver to U.C. Berkeley Mass Spectrometry Facility, c/o Dr. Ulla Andersen, B207 Stanley Hall, Berkeley, CA 94720. You will need to pay for the postage or deliver it yourself for free.

Analysis (½ class period about 1 week later)

1. Within a week, you should receive an e-mail with your students' results. They can determine the experimental caffeine concentration in their soft drink by looking at the gas chromatograph and mass spectrum. They compare the ratio in sizes of the abundance peaks of normal caffeine (MW 194) with that of the abundance peak for the known concentration of deuterated caffeine (MW 197).
2. Compare your students' experimental values to the book values for caffeine content in soft drinks (see PDF handout titled caffeine_content_drinks.pdf).
3. Sample answers to the Calculations and Analysis questions for teacher reference are in the document titled "Caffeine Lab Answers.pdf".

California State Science Standards that can be applied in this lesson:

Chemistry - Grades 9 Through 12 Science Content Standards

Chemical Bonds

2. Biological, chemical, and physical properties of matter result from the ability of atoms to form bonds from electrostatic forces between electrons and protons and between atoms and molecules. As a basis for understanding this concept:
   e. Students know how to draw Lewis dot structures.
   f.* Students know how to predict the shape of simple molecules and their polarity from Lewis dot structures.

Solutions

6. Solutions are homogeneous mixtures of two or more substances. As a basis for understanding this concept:
   a. Students know the definitions of solute and solvent.
   b. Students know how to describe the dissolving process at the molecular level by using the concept of random molecular motion.
   c. Students know temperature, pressure, and surface area affect the dissolving process.
   d. Students know how to calculate the concentration of a solute in terms of grams per liter, molarity, parts per million, and percent composition.
   f.* Students know how molecules in a solution are separated or purified by the methods of chromatography and distillation.

Investigation & Experimentation - Grades 9 Through 12 Science Content Standards

1. Scientific progress is made by asking meaningful questions and conducting careful investigations. As a basis for understanding this concept and addressing the content in the other four strands, students should develop their own questions and perform investigations. Students will:
   a. Select and use appropriate tools and technology (such as computer-linked probes, spreadsheets, and graphing calculators) to perform tests, collect data, analyze relationships, and display data.