# How Much Caffeine Is in My Soft Drink?
## Determination of Caffeine Content in Soft Drinks Using GC-MS

**Objectives:**

- To use chemical separation techniques to detect caffeine in soft drinks.
- To apply knowledge of solubility, molecular polarity, and density to explain separation techniques.
- To determine caffeine content in soft drinks by analyzing gas chromatography/ mass spectrometry data.

**Introduction:**

Caffeine is added to many soft drinks and energy drinks. Soft drinks differ from energy drinks in that soft drinks are regulated by the FDA and are limited in how much caffeine they can contain, whereas energy drinks are not. Some of the effects of human consumption of caffeine are well known to most high school students. From the National Institutes of Health: Excessive caffeine intake can lead to a fast heart rate, excessive urination, nausea, vomiting, restlessness, anxiety, depression, tremors, and difficulty sleeping. The effect of caffeine on health has been widely studied. In particular, the effects of caffeine on fibrocystic breast disease, heart and blood vessel disease, birth defects, reproductive function, and behavior in children has been closely examined.[1]

In this experiment, we will use reagents commonly found in a high school chemistry laboratory to separate pure caffeine from a soft drink or energy drink into a solvent suitable for GC/MS analysis. Most solvents commonly used in caffeine isolation as carcinogenic. However, in this experiment, we will use 70% rubbing alcohol (a.k.a. isopropanol or 2-propanol) since it is easily obtainable and safe to use in a high school laboratory. The caffeine will be isolated using knowledge of density, molecular polarity, and solubility.

Using GC-MS, you will determine the amount of pure caffeine extracted from your soft drink. Deuterated caffeine (MW 197) will be used as an internal standard, or a similar compound with a known concentration used in comparison to calculate the unknown concentration of pure caffeine (MW 194). Deuterated caffeine is structurally similar to pure caffeine, except that it contains 3 atoms of deuterium, or a hydrogen atom with an extra neutron. The relationship is directly proportional between concentration and the area under the abundance peak in a gas chromatograph. The equation is:

$$\frac{C_1}{C_2} = R \times \frac{A_1}{A_2}$$

where c = concentration, "R" = response factor (or the slope of the calibration standard line), and A = area under the abundance peak. The "R" value for deuterated caffeine was determined in the lab to be: 1.18.

If you know the concentration of one substance and have the areas under each peak for both substances, you can solve for the concentration of the second substance.

**Pre-Lab Assignment:**

On a clean sheet of paper, do the following: Draw a flowchart of the procedure. Then, answer the following questions, using your textbook or the Internet:

1. Is this caffeine separation based on chemical or physical properties? Explain.
2. Is a soft drink an element, a compound, or a mixture? Explain.
3. In your own words, describe the general principle that chemists use to perform chemical extractions.
4. Draw the Lewis structures for water, 2-propanol (or isopropanol), and caffeine.
5. Quantitatively compare the densities of isopropanol and water. Based on this information, predict what will happen when they are mixed together.
6. Based on molecular structure, explain why caffeine has greater solubility at a given temperature in isopropanol than in water.
7. Describe a few similarities and a few differences between caffeine and deuterated caffeine (caffeine-$d_3$).

**Materials for each group:**

**Apparatus**

- 25-mL beakers (2 needed)
- 1000 $\mu$L automatic pipets with disposable tips (1 per group)
- Small test tube with stopper (can hold around 5 mL)
- Test tube rack
- Glass stirring rod
- Electronic balance and weighing paper
- Spatula
- GC-MS Autosampler 1.5-mL microvial
- 100 $\mu$L automatic pipet with disposable tips (1 for instructor only)

**Reagents**

- Soft drink or energy drink (1 mL or ~1/30 fl. oz.)
- 70% Rubbing alcohol, isopropanol $CH_3CHOHCH_3$ in water (15 mL total)
- Anhydrous sodium carbonate, $Na_2CO_3$ (~0.15 g)
- Sodium sulfate, $Na_2SO_4$ (~0.10 g)
- Deuterated caffeine in 70% rubbing alcohol, 1 mg/mL (100 $\mu$L)

**Recommended Beverages (groups each choose one to bring in and test):**

1. Coca-Cola Classic
2. Diet Coke
3. Dr. Pepper
4. Jolt
5. Mountain Dew
6. Pepsi (Regular)
7. Diet Pepsi
8. Monster
9. Red Bull
10. Shasta Cola

**Notes:**

* Be aware there may be caffeine-free variations of these soft drinks. All brands used for this experiment should be the caffeinated version.

## Experimental Procedures:

**Preparation**

1. Prepare your soft drink by pouring at least 5 mL into a clean beaker. Use a glass stirring rod to liberate as much carbon dioxide from the soft drink.
2. Have your instructor pipet 100 $\mu$L of deuterated caffeine into a small test tube. Deuterated caffeine is what you will use to compare the amount of pure caffeine in your soft drink.
3. Pipet exactly 1.000 mL of soft drink into the small test tube and carefully add about 0.15 g of anhydrous sodium carbonate. Stir with a clean glass stirring rod to dissolve all of the sodium carbonate. Sodium carbonate makes some of the components in soda extremely soluble in water.

**Extraction**

*Pitfalls for Steps 4-8: If during any of the extractions an emulsion forms (when the liquids become mixed together and don't separate, sometimes has bubbles), let the tube stand for a few minutes after gently stirring the emulsion with the tip of your pipet.*

4. Add 500 $\mu$L of 70% rubbing alcohol to the test tube. Cap and invert gently 2-3 times, venting after each inversion to avoid pressure buildup. Allow the mixture to stand 5 minutes and separate into two layers.
5. Use a pipet to remove the top layer of isopropanol extracts from the tube into a clean beaker. If separate layers do not appear after 5 minutes, continue onto the next step without withdrawing any liquid.
6. Add a second 500 $\mu$L of 70% rubbing alcohol to the test tube. Cap and invert gently 2-3 times, venting after each inversion to avoid pressure buildup. Allow the mixture to stand 5 minutes and separate into two layers.
7. Remove the top layer of isopropanol extracts from the test tube and add to the extract beaker used in Step 5.
8. Add a third and final 500 $\mu$L of 70% rubbing alcohol to the test tube. Cap and invert gently 2-3 times, venting after each inversion to avoid pressure buildup. Allow the mixture to stand 5 minutes and separate into two layers.
9. Remove the top layer of isopropanol extracts from the test tube and add to the extract beaker used in Steps 5 and 7.
10. Add about 0.10 g of sodium sulfate to the combined isopropanol extracts in the beaker. Swirl gently. Allow the mixture to stand for at least 5 minutes so the sodium sulfate can absorb any excess water in the isopropanol.
11. Being careful not to withdraw any sodium sulfate, transfer 1 mL of the extract solution to a 1.5-mL GC/MS Autosampler microvial. Your instructor will submit your vials to U.C. Berkeley's Mass Spectrometry Facility for analysis.

*Waste Disposal:*

All remaining products can be discarded down the drain with plenty of water. Wash out glassware with warm, soapy water.

**Calculations – show your work:**

1. Calculate the mass in $\mu$g of deuterated caffeine you pipetted into your sample in Step 2 of the procedure.
2. Calculate the mass in $\mu$g of pure caffeine in your sample.
3. Calculate the mass in mg of pure caffeine in your sample.
4. Calculate the mass in mg of pure caffeine if you had 1 fl. oz. of your sample. [Hint: 1 fl. oz. = 29.5735296 mL]
5. Calculate the mass in mg of pure caffeine in a 12 fl. oz. can of your sample.
6. Calculate percent error.

**Analysis and Conclusions:**

1. Explain in terms of chemistry how density, molecular polarity, and solubility each played a role in this separation experiment.
2. If you had the opportunity to do this experiment again, how would you change the procedure or your methods to reduce error? Explain why.
3. Research GC-MS. Why is GC-MS a useful tool for distinguishing between pure caffeine and deuterated caffeine in the same sample, like in your experiment?