

Determination of the Caffeine Extraction Profile of Brewing Coffee

Before lab:

1. Write a procedure for the preparation of a 1000 ppm caffeine stock solution
2. Calculate the volumes needed to dilute the 1000 ppm caffeine stock solution in order to achieve 0, 100, 250, 400, and 500 ppm standards
3. Write a procedure **similar** to the examples shown below describing what you want to do.

Introduction:

When coffee or tea is prepared, hot water is used to extract flavor components and other molecules, such as caffeine are also extracted caffeine. Caffeine (1,3,7-trimethylxanthine, FM=194.19g) is slightly soluble in water and is a central nervous system stimulant with a bioavailability of 99% (CRC and wikipedia). The onset of action is typically 45 minutes and the typical half-life inside the body is 3-7 hours. Typical caffeine concentration in beverages and common foodstuffs is shown in **Table 0-2** (Harris, 2005).

The purpose of this lab is to experimentally determine the caffeine extraction profile (a plot of mass of caffeine extracted versus time) for the brewing of caffeinated beverages. You will develop your own procedure to determine caffeine concentration for the brewing or steeping profile of your beverage of choice.

After sample collection, concentration of caffeine will be detected and quantified using High-Performance Liquid Chromatography (HPLC). The HPLC is used to separate the components of samples, in this case coffee or tea, into individual components using a chromatographic column. These individual components are detected using a UV-Visible spectrophotometer or mass spectrometer. The UV-Visible spectrum of caffeine shows substantial absorbance at 272 nm. The molar absorptivity at 274 nm is approximately $2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ in ethanol solution. This wavelength is monitored and the caffeine standard demonstrates a retention time of 7.3 minutes (**Fig. 1**). The mass spectrometry detector also detects caffeine at 7.3 minutes and shows a distinctive mass spectrum pattern (**Fig. 2**). In actual samples, the matrix is more complex, this results in a more complicated chromatogram. The mass spectrometry and UV-Visible chromatograms are different, since the UV-Vis chromatogram results for analyte molecule absorbance at 272 nm while the mass spectrometry chromatogram counts the total number of

TABLE 0-2 Caffeine content of beverages and foods

Source	Caffeine (milligrams per serving)	Serving size ^a (ounces)
Regular coffee	106–164	5
Decaffeinated coffee	2–5	5
Tea	21–50	5
Cocoa beverage	2–8	6
Baking chocolate	35	1
Sweet chocolate	20	1
Milk chocolate	6	1
Caffeinated soft drinks	36–57	12
Red Bull	80	8.2

a. 1 ounce = 28.35 grams.

SOURCES: http://www.holymtn.com/tea/caffeine_content.htm. Red Bull from <http://wilstar.com/caffeine.htm>.

Harris, *Quantitative Chemical Analysis*, 8e

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analyte ions reaching the detector as a function of time.

Figure 1: Total ion count chromatogram and mass spectrum of the caffeine standard as detected by the mass spec detector.

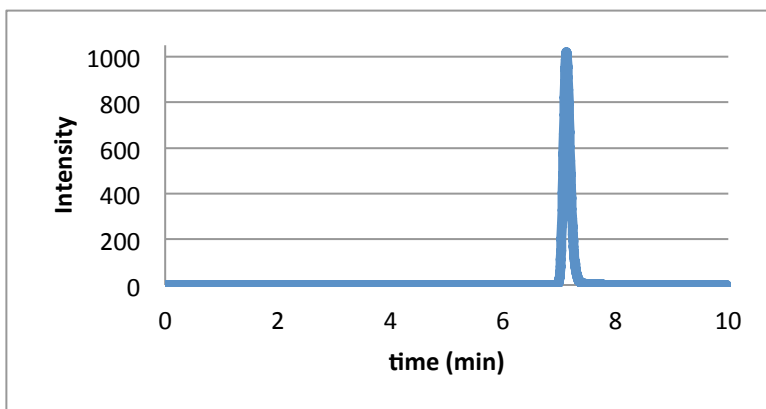
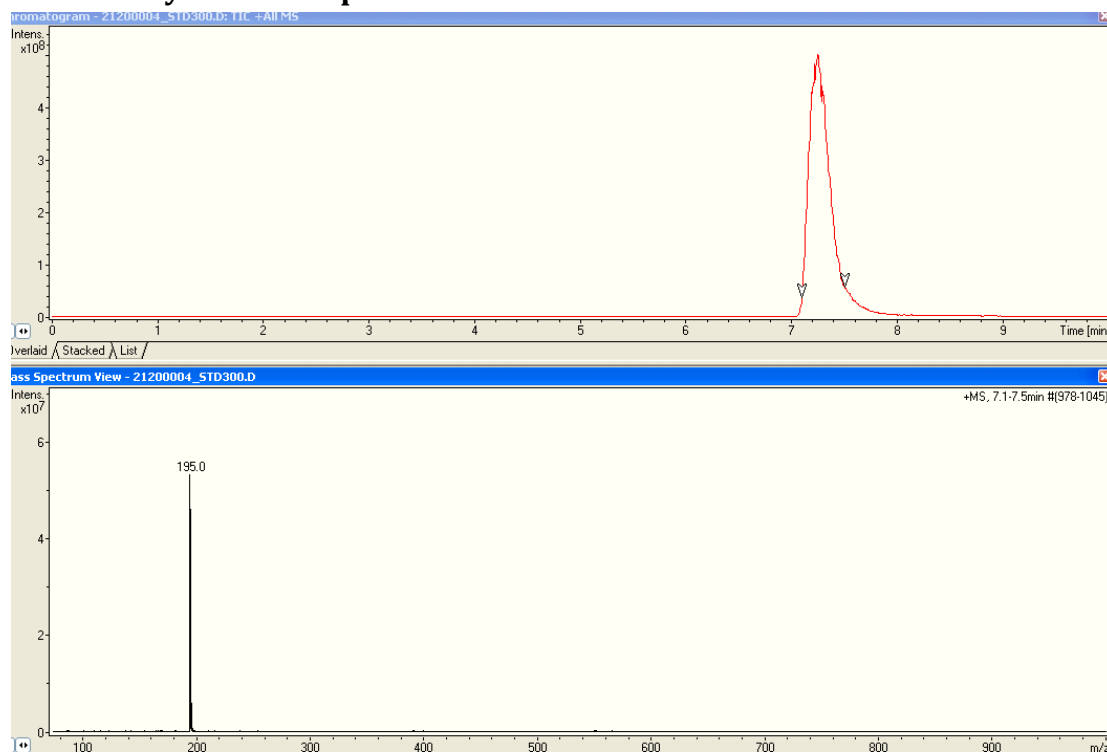


Figure 2: Total ion count chromatogram and mass spectrum of the caffeine standard as detected by the mass spec detector.



Procedure: Be creative!

You can examine the caffeine extraction profile for anything (coffee, espresso, tea, whatever) with a caffeine content that will change as a function of time within 15 or so minutes. If you need specialized equipment or supplies (tea, coffee, etc) you need to bring them with you to lab. If you bring your own coffee maker or similar we will perform the experiment outside lab to prevent contamination.

Complete procedures will include:

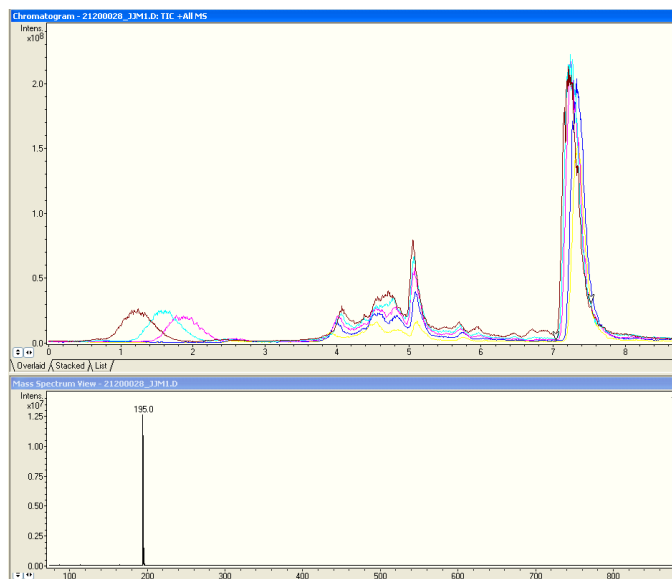
1. Brewing or steeping conditions (volume of solution, mass of coffee or tea, temperature of water, etc).

- Time points at which samples will be taken. Caffeine extraction is a relatively fast process and the most interesting changes generally occurs during the first 2-3 minutes. We can run 5 samples per group on the HPLC.
- Filter samples using a 0.45 micron syringe filters and fill HPLC vials. Be sure to label your vials using some system that is unique to your group (remember the instructor and TA will have many vials to keep track of, so make it easy for them to keep your samples grouped together and for you to determine the sample order).

Example 1: Determine the Caffeine Elution Profile of a Brewed Coffee

- Start brewing 4 cups of coffee with the coffee pot and 20g coffee grounds
- Collect five aliquots of coffee at 30 seconds, 60 seconds, 90 seconds, 120 seconds, and after brewing has been completed.
- Filter each sample with their own individual 0.45 micrometer filter and syringe.
- Fill up an HPLC vial with portions of each of the filtered samples
- Give the TA your labeled samples or take them to the HPLC.

Figure 3: Total ion count chromatogram and mass spectrum of the brewing tea as detected by the mass spec detector. Notice the caffeine peak seems relatively constant with time, but the flavor peaks (retention times ~4-6 minutes) grow substantially as a function of time.



HPLC instrumental procedure:

Samples were separated on an Agilent 1100 series High Performance Liquid

Chromatography equipped with a photodiode array UV-Vis detector and an Agilent LC/MSD Trap Mass Spectrometer detector. A 50:50 H₂O:methanol solvent mixture at 0.5 mL per minute mobile phase was passed through an Agilent Eclipse XDB-C18 column with 5 um particle size. The 5 uL of filtered sample was injected into the instrument and allowed to run for 10 minutes. The UV-Vis detector monitored 272 nm for a caffeine absorption signal while the MS detector caffeine peak appeared at 195 m/z.

Week 2, Calculations:

- A calibration curve will be generated by printing the calibration curve and sample chromatograms, cutting the peaks out of the paper, and weighing the peaks on an analytical balance. As long as axis are the same and the paper is uniform, the area under the curve is proportional to the mass of paper.

2. Plot a calibration curve (x axis- concentration caffeine, y-axis is mass of peak), find line of best fit and r-squared value. Use the line of best fit to determine the concentration of caffeine in your samples.
3. Plot the concentration of coffee as a function of time or aliquot. Make observations about how the concentration of caffeine changes as a function of time.

Discussion questions:

1. What observations can you make about brewing time versus caffeine content in your beverage of choice? How would you recommend to make the strongest cup of tea or coffee?
2. Comment on how the experiment worked. If you were going to redo the experiment, would you change the time intervals between samples?
3. Identify what you believe to be the largest source of error in your experiments and discuss how they would have affected your results.

Lab write-up:

1. **Abstract:** In 250 words or less, describe your purpose, what you did, what you found, if that was expected or surprising, and what it all means.
2. **Introduction:** Introduce topic and state why you are performing the experiment.
3. **Materials and Methods:** Prepare a trimmed down procedure in sentence form.
4. **Results:** tabulate your measurements and results; and briefly say what they mean.
5. **Discussion:** respond to the questions above in paragraph form (not Q1: answer, answer). Devote 1-2 paragraphs to discuss each question.
6. **Conclusion:** Write a short paragraph concluding what you learned.
7. **Group dynamics:** let me know how you feel your group worked together.
8. **Appendix 1- Calculations:** You can send me your spreadsheet.

References:

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CRC Handbook of Chemistry and Physics. CRC Press: Boca Raton, FL.

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Harris, D. C. Quantitative Chemical Analysis. 8th ed. W. H. Freeman: New York, 2010.

Beckers, J. L. The determination of caffeine in coffee: sense or nonsense? Journal of Chemical Education, 2004, 81 (1), 90-93.