Experiment 3: Determination of Caffeine in Beverages Using HPLC

CH3360: Instrumental Analysis, Plymouth State University

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Introduction:
Caffeine is a molecule that needs no introduction. Caffeine is my friend, and it is yours. For many, there is no need to look further than caffeine for proof of the existence of a loving and kind higher being. So, without further ado, here it is, along with its UV-Vis spectrum:

![Caffeine Structure](image)

\[
\text{Caffeine} \quad \text{C}_{8}H_{10}N_{4}O_{2}, \text{MW: 194.19 g}
\]

In this lab, you will use reverse-phase HPLC with a UV detector to quantify caffeine in various beverages. Most coffee, tea, soda, etc. beverages contain 300-600 mg/L (ppm) of caffeine. Columns and detectors in HPLC typically have an upper limit of separation/detection on the order of a few 10's of ppm before peak broadening and saturation of the detector occurs. A series of standards of pure caffeine will be prepared in this range and used to develop a standard curve. Samples will be diluted appropriately to bring the concentration of caffeine into the quantifiable range.

Equipment: Read through the procedures and make a list of the equipment you will need.

Safety Considerations: Read through the procedures and note any safety considerations.

Procedure: (students will work in groups of 2-3 to do the following):

A. Preparing mobile phase

1. The mobile phase for this will be a mix of water and methanol. Flasks of pure water and pure methanol, degassed using sonication, will be provided. The exact mix of the two solvents will be set with the HPLC equipment (see below).

B. Preparing standards

1. Accurately prepare 500 mL of 100 ppm Stock Solution of caffeine. Record the mass used to all the available digits and use volumetric glassware to achieve 4 significant figures of precision. Calculate the actual concentration. Note: Two groups may share the same Stock Solution.
2. Prepare a series of standards from the Stock Solution: 1, 5, 10, 20, 30, 40 and 50 ppm using available pipettes and 25, 50, or 100 mL volumetric flasks. Calculate the actual concentrations based on the concentration of Stock Solution. Note: To save time in lab, consider how to do this as part of your pre-lab work.
C. Preparing samples

1. Dilute your sample appropriately to get the concentration between 1 and 50 ppm, assuming it will start out in the 300-600 ppm range. Less than 1 mL of sample will actually be used, so use smaller volumetric glassware to prepare your solutions. *Note: To save time in lab, consider how to do this as part of your pre-lab work.*

D. Setting up the instrument

1. Use the following parameters:
   - Flow: 0.8 mL / min
   - Mobile phase composition: 50:50 methanol to water
   - Run time: 15 min
   - 20 μL injection
2. Consult the UV-Vis spectrum of caffeine and choose 4 wavelengths to observe
3. Prepare a sequence including
   - Each of your Samples run in duplicate
   - Each of your Standards run in duplicate
   - A clean-out run with 80/20 water MeOH for 10 min and 100% MeOH for 10 min
   - A shutdown run

E. Final Checks

1. Calculate the volume of mobile phase that will be required for all the runs. Do you have enough? Is there enough room in the waste container?
2. Is the UV lamp on and collecting data?
3. Are your samples in the autosampler in the correct locations?
4. Do you have enough "sample" for personal consumption while you wait for the analysis to complete?

Analysis (Lab Notebook)
The following must be completed in your lab notebook before you can turn in your Notebook Report:

1. Record the retention times, peak areas, and widths of peaks for caffeine in all your Standards and Samples.
2. Each Standard was run twice. Use the averages of the peaks areas for each Standard to set up a standard curve. Calculate the concentration of caffeine (in ppm) in your Diluted Sample. Calculate the concentration of caffeine (in ppm) in your original Sample. Calculate the mass (in mg) of caffeine that would be in 16 oz of your sample (seriously, who drinks only 8 oz of coffee?).
3. Calculate the number of theoretical plates in the present setup for caffeine.
4. Calculate the resolution factor between caffeine and its nearest neighbor in your sample.
Lab Report including Conclusions and Discussion

Your lab report is due by lecture on Wednesday,. Your report must be handed in BOTH electronically (via Moodle) and in hard copy form. See the document "LabReportGuide.pdf" on the course website (http://oz.plymouth.edu/~jsduncan/courses/2011_Fall/InstrumentalAnalysis) for guidelines on writing your report.

In addition to the Analysis performed in your notebook, include the following in your report (place them in the most relevant sections):

1. Comment on the effectiveness of this technique (HPLC) at quantifying caffeine. Include evidence from your data / analysis to back up your statements.

2. Are the parameters (mobile phase composition, flow rate, column length) sufficient to resolve the analyte of interest? Suggest a change in parameters that would improve resolution and/or analysis time.

3. This lab used a Standard Curve for quantification of the analyte. Discuss the limitations and assumptions in using this method. Suggest a way to overcome the limitations. Consider your Sample's chromatogram in this discussion.