High Pressure Liquid Chromatography (HPLC) Analysis of Caffeine in Tea.

Reading   Essay: *Caffeine* in Pavia (p. 96). Technique 21 in Pavia (5th edition). The procedure is similar to (*but not exactly the same as*) Experiment 51 in Pavia.

Background

The presence and amount of caffeine found in brewed tea can be measured using HPLC analysis. To determine the actual quantity of caffeine requires generating a calibration curves from known concentrations of caffeine. Using the calibration curve for pure caffeine, the concentration of caffeine in a caffeinated beverage, such as soda, coffee, tea, or any other "modern" caffeinated beverage, can be determined. We will analyze the same tea that was used in Experiment 13 and you will compare the amount determined by HPLC with the amount isolated by extraction.
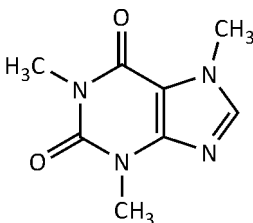
caffeine

Prelab

Include **Name**, **Date**, **Title**, **Purpose**, and **Outline of Procedure** (see below).

Procedure

Brew a cup of tea in the laboratory: measure 75 mL of deionized water using a graduated cylinder and add it to a 250 mL beaker. Using a hot plate, heat the liquid to a gentle boil. Weigh a tea bag (the same type used in Experiment 13) and record the mass in your notebook (note the average mass of the tea bag packaging is the same as for Experiment 13). Add the tea bag to the boiling water and "push" the bag to the bottom of the beaker with a test tube to make sure it is completely under the level of the liquid. Maintain a gentle boil for 5 minutes, then allow the beaker to cool to room temperature. Filter the cooled solution through a fluted filter paper using the powder funnel into a clean 125 mL Erlenmeyer flask. Rinse the beaker and tea bag with 10 mL of hot deionized water, then filter. Squeeze as much liquid as possible from the tea bag using the test tube and filter. Transfer the combined filtrate (quantitatively) to a 100.00 mL volumetric flask and then dilute to the mark (bottom of the meniscus). Cap the flask and mix well. Using a volumetric pipette and volumetric flask, dilute 10.00 mL of this solution to 250.00 mL, cap and mix well. Pour approximately 25 mL of this solution into a 100 mL beaker and using the provided syringe, filter a sample of this diluted sample through a 0.45 μm nylon filter directly into an autosampler vial for HPLC. Add your sample to the HPLC autosampler tray and add your name at the indicated slot number on the sign-up sheet.

Once the HPLC analysis is complete, locate the caffeine peak in the sample – use the retention time found in the caffeine standards (from the instructor) - and integrate the peak to obtain the corresponding peak area. Print out the data using the Caffeine Report sheet on the computer (you will receive instructions on the use of the HPLC in the laboratory).

To Complete the Experiment – Partial Report

Prepare a *Calibration Curve* for determination of caffeine concentration by plotting chromatogram peak area (y-axis) vs. the concentration of caffeine in ppm (x-axis) for the four *Standard Solutions*, with concentrations of 5.0, 10.0, 20.0 and 40.0 ppm (these will be provided to you). For aqueous solutions, 1 ppm = 1 mg/L. Use a graphing program on the

computer (e.g., Excel or OpenOffice Calc (which is free)) to prepare your curve and display the best-fit line (least-squares analysis) of the data and also display the equation of the line along with the correlation (R^2).   For your sample, determine the area for the caffeine peak and *interpolate* the calibration curve to find the concentration in the sample that was injected. This concentration will be in ppm.

Calculate the concentration (ppm) of the original sample before dilution, then using the original volume of solution ("brewed" tea) that was prepared, calculate the total caffeine (mg) that was "extracted" in the brewed tea. Using the weight of the tea that you used, calculate the wt% of caffeine in the dry tea and then compare this amount of caffeine with what you obtained in Experiment 13. (Note that the weight of tea in the bag is obtained from the mass of the dry tea bag minus the average weight of the tea bag packaging obtained in Experiment 13) Write out all of your calculations and commentary in your notebook.

Finally, convert the concentration of caffeine you found in the tea sample from units of ppm to mg/oz (note that 1 US fl. oz. = 29.57 mL). Compare your result with the values shown in the Table at the top of p. 98 in Pavia (5th edition).
Show all of the calculations and conversions in your notebook. Be sure to include your printed HPLC chromatogram (raw data) with your completed experiment.

For this experiment turn in the following, in this order:

1. Cover page
2. Conclusion – summarize the results from both experiments and compare the wt% of obtained in each experiment.
2. Notebook pages – both experiments
3. Calibration curve (with all units, title, equation for the line, R^2)
4. HPLC Chromatogram (report from HPLC)