The Determination of Caffeine by High Performance Liquid Chromatography (HPLC)

Introduction:

This experiment is designed to introduce the basic use of an HPLC for performing a separation and quantitative determination. As all good students know, caffeine plays an important role in modern life, particularly in the form of beverages. The goal of this laboratory experiment is to determine the concentration of caffeine in one of your favorite caffeinated beverages.

Required Chemicals and Solutions:

1. A caffeinated beverage
2. HPLC grade methanol (for mobile phase)
3. 18 MΩ resistive water
4. Caffeine aqueous stock standard solution at 1000 µg/mL (ppm).
5. 4-5 Standard aqueous caffeine solutions ranging from 1-40 µg/mL

Sample Preparation:

After obtaining a sample or samples of a beverage containing caffeine (or perhaps decaffeinated). If the material is one which is solid, it must first be dissolved into solution. As an example, if coffee is of interest, one must first brew the coffee or dissolve the instant coffee in solution to create a typical serving of the beverage. However, unlike making the beverage to drink, you should weigh the solid and dissolving it to a known volume (as per a standard serving). If you have a carbonated beverage, you must degas the beverage before preparing a sample for analysis.

Once the gross sample has been readied, sample preparation can begin. The beverage must be filtered to remove particulates using a syringe filter with no larger than 0.45 µm pore size. Dilute the filtered beverage with water to prepare a sample whose caffeine concentration falls *within your calibration range*. For your results to have any utility, your standards must bracket your sample. The ingredient label (or the internet) may be a good source of information to decide on a dilution scheme. Prepare 3 replicate samples.

Analysis:

All standards and replicate samples should be analyzed using reverse phase HPLC with a filtered and degassed methanol/water mobile phase and absorbance detection. Quantitation will be done by comparison with a calibration curve prepared using known caffeine standards run under the same conditions as the samples. Before preparing the calibration curve, separations conditions must be optimized to ensure that the caffeine peak is adequately resolved from other components in the sample in minimal time. Your instructor may choose to provide optimum conditions for you, or may ask you to optimize the separation yourself. Optimization of the method involves changing parameters like mobile phase composition and flow rate until the required separation quality is reached.

Prior to beginning the separation, a detection wavelength must be chosen to give optimum sensitivity for your caffeine analyte. Using a UV-Vis spectrophotometer, collect a qualitative spectrum of caffeine and determine the optimum wavelength for the LC detector.

Once a detection wavelength has been set, separations can begin. Choose an initial flow rate in the range of 1.0-1.5 mL/min and an initial mobile phase composition in the range of 80:20

to 60:40 methanol:water. Sample sizes should be consistent and should be within the range of 5-20 µL. Be sure to record all conditions. Allow at least 5 minutes for the column to equilibrate to the new conditions and inject your highest concentration caffeine standard (40 ppm). Note the retention time (t_r), peak height (H) and peak area (A) for the caffeine peak. Next, inject your beverage sample and note the quality of the separation of the caffeine peak from other components in the sample. If the caffeine peak is completely resolved, record its t_r, H, and A. If the caffeine peak in your sample is larger than that in your 40 ppb standard, the beverage sample is too concentrated and must be diluted quantitatively to bring it within your calibration range.

Try altering the flow rate of the mobile phase and observe the impact on the retention time of the caffeine as well as the column efficiency (the HETP). Also, try altering the mobile phase composition in the range of 80:20 to 60:40 methanol:water, again noting the impact on retention behavior and column efficiency. Allow at least 5 minutes for the column to equilibrate to the new conditions after making any changes. Based on your observations, select the set of conditions that gives a quality separation for caffeine in minimum time.

Using your optimum conditions, run each of your standards and samples, recording t_r, H, and A for the caffeine peak for each run. Make triplicate injections for at least one of your standards to explore the run-to-run reproducibility of the separation.

Results:

Using calibration curves created by both peak areas and peak height, determine the concentration of caffeine in the beverage you used for a sample. Compare this result to that expected for the sample you chose. (Remember, this requires you to back-calculate to the original concentration of your sample; thus knowing your dilution trail precisely becomes critical).

It is also crucial to report the precision of the analysis and ultimately the confidence limits. In addition, you should report the % error by comparison of your calculated analyte concentration to that expected for your beverage. Finally, with knowledge of your precision and the calibration curve you obtained, you should be able to determine an approximate limit of detection for the method of analysis, under the experimental conditions you used for the measurement.

What determinate errors might have played a role in impacting the accuracy of your results? What factors might have been the greatest contributors to the precision you obtained during this analysis.