Quantitative Analysis of Caffeine and Benzoate in Soda Pop by RP-HPLC

Background

This project will use reverse phase high performance liquid chromatography (RP-HPLC) with ultraviolet absorption detection for the determination of caffeine and benzoate in soda pop. It will help to review the technique of liquid chromatography in your instrumental analysis text.

Reverse phase HPLC is the most popular mode of analytical liquid chromatography in use today. In simple terms, the stationary phase is less polar than the mobile phase. This mode works well for separating solutes of low to moderate polarity because of the greater attraction of the stationary phase for these solutes relative to the mobile phase.

UV absorbance detection is probably the most common detection mode used. The detector used in the lab is a variable wavelength detector which allows the operator to choose the wavelength to monitor. Solutes passing through this beam that absorb at the specified wavelength attenuate the intensity of the light reaching the detector photocell. This results in a voltage signal which is read by a strip-charter recorder or integrator. The height of the chromatographic peak is directly proportional to the solute concentration in the detector at that particular moment. Thus, a calibration curve can be obtained simply by plotting peak height vs. concentration.

Column Care

When properly maintained, HPLC columns have a useable lifetime of several hundred separations.

- Use only HPLC grade solvents
- Filter and degas all mobile phases
- Filter samples
- Equilibrate the column with mobile phase for at least 30 minutes whenever changing mobile phase composition or flow rate.
- Wash the column with pure water for 30 minutes when you are through for the day.

HPLC Part I

Modern HPLC instruments such as the Thermal Separation Products (TSP) HPLC you will be using to analyze caffeine and benzoate in soda pop look rather complicated. Most are computer controlled, have multi-channel pumps, integral solvent degassers and gradient mixers, auto samplers and two or more types of detector.

Although convenient for the user, these 'extras' are not really necessary for producing high quality separations. A pump, detector and a visual output are all that is really needed.

Your assignment for part I is to assemble our 'bare bones' HPLC and produce a chromatogram showing the separation of a standard mixture of caffeine and benzoate.

Notes

Use a mobile phase of 50:50 methanol:water buffered to pH 3 with 10 mM phosphate.

Filter and degas the mobile phase.

Prepare a solution of 0.10 mg/ml caffeine and 0.30 mg/ml benzoate in diH_2O.

Be sure to purge all lines with mobile phase before attaching the column.

Use a detection wavelength of 254 nm.

Allow the column to equilibrate for at least 30 minutes at 1ml/min before performing the separation.

After the separation wash the column with 100 % water for 30 minutes at 1 ml/min.

HPLC Part II: Quantitative Analysis of Caffeine and Benzoate in Soda Pop

Objective

- To determine the effect of mobile phase composition on the separation of caffeine and benzoate.
- To quantify the concentrations of caffeine and benzoate in soda pop.

Instrumentation

Thermal Separation Products HPLC equipped with a UV3000 UV detector.

Computer Software

ChromQuest HPLC instrument control and data analysis software.

NOTE: You may analyze as many different brands of soda pop as you wish. The containers should be left open for at least a couple of days or otherwise treated to remove the CO_2.

Experimental

The TSP instrument start-up needs to be performed in a certain way. Please ask your TA to show you the start-up procedure before beginning.

Set up a folder for your group in the E:\CH4212 directory. Please save all method and data files to that folder.

Do not attach the column until all lines have been purged.

Never remove tubing from the solvent reservoirs while the pump is running. It can be difficult and time consuming to purge the pump.

The Windows NT based ChromQuest software will look familiar but it is not particularly intuitive. Ask your TA to show you how to create, save and load method files, check the instrument status, start a run and print the chromatogram.

<u>Effect of Mobile Phase Composition</u>

Run the standard mixture from part I using a mobile phase composition of 50:50 methanol:buffered water.

Now run the mixture using two different mobile phase compositions. How does the mobile phase composition effect the separation?

Calibration Curves

You will need to prepare calibration curves for both caffeine and benzoate in order to quantify their concentrations in the unknown.

Using a mobile phase composition of 50:50 methanol:buffered water, prepare and run at least three different concentrations each of caffeine and benzoate (run separately). The concentrations used for the standard mixture from part I is a good place to start.

Unknown

Run your soda pop samples and calculate the mass/volume percent of caffeine and benzoate.

Calculate the total amount of caffeine and benzoate in the full can of soda.

Report

In your report include:

The chromatogram from part I

A sketch of the assembled instrument from part I showing the components and connections.

All chromatograms from part II

Calibration curves from part II

Sample calculations