

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

DETERMINING CAFFEINE CONCENTRATIONS IN SOFT DRINKS

(Revised: 5-18-93)

INTRODUCTION

Chromatography is a separation technique that was first used by the Russian botanist Mikhail Tsvet. Around the turn of the century he was attempting to separate a mixture of liquids by pouring them into a column of powdered chalk. After a while he noticed bands of colors corresponding to the different components of the mixture. Tsvet named the technique chromatography from the Greek for "color writing". Despite the name, chromatography can be applied to uncolored substances as well as those that are colored.

There are several separation methods that fall under a general category known as chromatography. All of the methods have one common principle that distinguishes them from other physical and chemical methods of separation. This common feature is both a **stationary** and **mobile phase**. These two phases are incapable of being mixed, and come in contact with the sample that is to be separated, numerous times.

The sample is carried through the system by the **mobile phase** and the interactions that occur are due to the different physical and chemical properties of the sample components. Separation is then a result of these different properties which affect the rate at which the components pass over the **stationary phase**. This rate, or **retention time (R_t)**, is determined as a result of the UV detector "seeing" each component as it elutes from the column. It will then record these different substances as different peaks relative to the rate which they elute out of the column. The size of the peak indicates the amount of the component in the sample.

The High Performance Liquid Chromatograph (HPLC) instrument that you will use for this laboratory exercise follows these same principles. Your HPLC has a column that has been carefully packed with C-18 particles that are less than 10 micrometers in diameter. This is the **stationary phase** and allows this instrument to provide unprecedented resolution and efficiency. Since the particles in the column are so small, it is necessary to pump the **mobile phase** (a 30% methanol solution) through the column at a high pressure (between 3500-3800 psi). The pump keeps a precise flow rate so that the peaks of each sample component can be measured in time. This is then compared to **prepared standards** of the particular component of interest. The peak area of the component of interest (caffeine) will then give us a quantified mg/ml value for each sample tested through the use of a calibration curve.

PURPOSE

In this experiment you will identify the caffeine peaks of various soft drinks by determining

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

their the retention time. Then each soft drinks' caffeine peak area will be calculated and used in a calibration curve of previously run caffeine standards to determine each of the soft drink's "kick".

SAFETY AND PROCEDURAL CONSIDERATIONS

1. Safety goggles should be worn during the use of the HPLC.
2. Syringes are very delicate. They must be handled with extreme care. Make sure you completely understand your teacher's instructions on its use.
3. The only adjustment that will be made of the instrument is the AUFS* setting. Be sure to tell your teacher that you are making such a change as directed by your procedure.
4. It is very important that the soft drinks that are tested are diluted 1:1 (50% soft drink solution). If this is not done, sugar can clog the injection port and column.

* AUFS - Absorbance Units Full Scale - Therefore, 1.0 AUFS means that an absorbance unit of 1.0 would register full scale.

PRE-LAB QUESTIONS

1. Develop a Vee diagram for your portfolios.
2. In your portfolios, write an essay that discusses the following:
 - a. how HPLC is used as a separation technique.
 - b. what the purpose is of the mobile phase.
 - c. what the purpose is of the stationary phase.
 - d. what the purpose is of the caffeine standards.
 - e. what is meant by retention time.

MATERIALS

Equipment

1. HPLC instrument
2. 50 microliter syringe
3. 5 - 10 small test tubes*
4. Nylon acrodisc filters
5. 1, 5, or 10 ml pipet*
6. C - 18 column
7. 10 mL vial*

Substances

1. soft drink samples
2. caffeine standards (5 - 8) **
3. mobile phase solution
4. distilled water *

* - These items will not be provided by the Chemobile.

** - Only the solid caffeine necessary to prepare the standards will be provided.

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

PROCEDURE

Developing the caffeine standards (option #1)

1. The caffeine standards can be handled in one of two ways depending on time and availability. The first option is to provide the students with a .20 mg/ml stock solution of caffeine that will be diluted into the following:

Suggested concentrations for the standards are:

- a. 0.02 mg/ml (AUFS setting is .05)
- b. 0.04 mg/ml (AUFS setting is .2)
- c. 0.06 mg/ml (AUFS setting is .2)
- d. 0.08 mg/ml (AUFS setting is .5)
- e. 0.10 mg/ml (AUFS setting is .5)
- f. 0.12 mg/ml (AUFS setting is .5)
- g. 0.16 mg/ml (AUFS setting is 1.0)
- h. 0.20 mg/ml (AUFS setting is 1.0)

(The chart speed setting is best at 5 cm/min for standards and soft drink samples.)

2. Each group of three will be assigned a caffeine standard (a - h) to make from the stock solution provided. Have the group calculate their dilution procedure and decide how this will be done with the pipet they will be using. At least one milliliter is needed, 10 ml is the easiest to make. Group "h" can go right to the HPLC.

3. Each group must then filter their standard using a nylon acrodisc syringe filter. Be sure to put the filter on the syringe only **AFTER** you have drawn up the solution. Filter the sample into a 10 mL vial. (For further information see "**HPLC Instructions for Operation**").

4. Draw up 50 microliters of your filtered sample and discard into a waste container. Do this twice.

5. Draw up a sample to the 50 microliter mark making certain no air bubbles are in the syringe.

6. Make sure the injection lever is in the load position, that you have the correct AUFS

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

setting, and the recorder is ready.

7. Inject your sample while in the load position, then move the injector lever to the inject position. Push the event button and leave the syringe in the injection port.

8. On your chart paper record the following:

- identity of sample
- chart speed
- AUFS (**range**) value

9. After the peaks have been recorded, return the injection lever to the load position and remove the syringe. Clean the syringe with distilled water.

10. Each group should determine the **retention time, R_t** , of the caffeine. Record your values in DATA TABLE 1. This is the time from the event mark until the middle of the peak. For instance, if the chart speed is 2.5 cm/min and the distance between event mark and the middle of the peak is 4.5 cm, then the **retention time** is 1.8 min.

11. Each group should now determine the **adjusted peak area** for your caffeine standard. Your teacher will instruct you to use either the "triangulation" method or the "cut and weigh" method. Record your values in DATA TABLE 1.

A.) **"triangulation" method**

- i.) Assume that the peak is a triangle.
- ii.) Determine the relative peak area as :
$$\text{Area} = \text{height} \times \text{width at } 1/2 \text{ height}$$
- iii.) Multiply the relative peak area x the **Range (AUFS)** value to calculate the adjusted peak area

B.) **"cut and weigh" method**

- i.) Cut out the standard peak and record the mass in mg.
- ii.) Cut out a 1 square cm piece of the chart paper and record its mass in mg.
- iii.) Divide the mass of the peak by the mass of the cm^2 to get the relative peak area.
- iv.) Multiply the relative peak area x the **Range (AUFS)** value to calculate the adjusted peak area.

12. Each group should report to the class their adjusted peak area for the caffeine standard

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

they ran. Also, each group should report their retention time (R_t) for their caffeine peak, and all of this should be recorded in Data Table 1.

Developing the caffeine standards (option #2)

1. Using the provided photocopies of caffeine standards for each concentration, each group of three should determine the **retention time, R_t** , and the adjusted peak areas.
2. Calculate the adjusted peak area for the caffeine peak as described in **Option #1, #11**. Each group should report to the class their adjusted peak area for the caffeine standard they studied. Also, each group should report their **retention time** for their caffeine peak, and all of this should be recorded in Data Table 1.

Preparing the soft drink samples

Each group of three will be assigned a soft drink.

1. First each soft drink must be degassed. This is done by simply bringing a 10 ml sample to a boil or allowing it to sit in an open container overnight.
2. Next each sample should be diluted 1:1. (50% SOFT DRINK SOLUTION)
3. Filter the sample using a nylon acrodisc syringe filter so that one ml is available for testing. Remember to draw the solution into the syringe before putting on the filter.
4. Follow the injection procedures in **Option #1, #'s 4 - 9**.
5. On your chart paper record the following:
 - identity of sample
 - chart speed
 - AUFS (**range**) value (start by trying AUFS = .5, and adjust if necessary)
5. Determine from the retention time which peak is caffeine.
6. Calculate the adjusted peak areas for your soft drink peak as you did in **Option #1, #11**. Record it into Data Table 2.

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

7. Report this value to the class.

Name _____
Class Period _____

Identity of Soft Drink _____

**DATA TABLE 1 FOR STANDARDS
(Triangulation Method)**

Standard Conc. (mg/mL)	R _t (min)	Peak Height (cm)	Width at 1/2	Relative Peak Area	Range (AUFS)	Adjusted Peak Area
0.01						
0.05						
0.08						
0.10						
0.13						
0.15						
0.18						
0.20						

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

Name _____

**DATA TABLE 2 FOR SOFT DRINK
(Triangulation Method)**

Soft Drink	R _t (min)	Peak Height (cm)	Width at 1/2 Peak Height (cm)	Relative Peak Area (cm ²)	Range (AUFS)	Adjusted Peak Area (cm ²)	Conc. From Graph (mg/mL)	Actual Soft Drink Conc (X 2) (mg/ml)

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

Name _____

Identity of Soft Drink _____

Class Period _____

**DATA TABLE 1 FOR STANDARDS
(Cut and Weigh Method)**

Caffeine Standard Conc.	R _t (min)	Peak Mass (mg)	Mass of cm ² Piece	Relative Peak Area	Range (AUFS)	Adjusted Peak Area
0.01						
0.05						
0.08						
0.10						
0.13						
0.15						
0.18						
0.20						

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

Name _____

**DATA TABLE 2 FOR SOFT DRINKS
(Cut and Weigh Method)**

Soft Drink	R _t (min)	Peak Mass (mg)	Mass of cm ² Piece (mg)	Relative Peak Area (cm ²)	Range (AUFS)	Adjusted Peak Area (cm ²)	Conc. From Graph (mg/mL)	Actual Soft Drink Conc (x 2) (mg/ml)

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

DATA ANALYSIS

1. Construct a calibration curve using the data from Table 1. Plot the concentration on the x-axis and the adjusted peak area on the y-axis. Connect the points with a best fit line.
2. Using the retention time (R_t) from Data Table 1, determine which peak is caffeine from your soft drink chromatogram.
3. Using your calibration curve, find your adjusted peak area on the y-axis for the soft drink you ran. Find the value for concentration which corresponds to this peak area. You must multiply this by 2 since we diluted the soft drink 1:1. Report this to the class and record these in Data Table 3.
4. Do a percentage of error calculation for your soft drink and report this to the class. (The experimental value is what you have calculated from the calibration curve. The theoretical value is the companies' caffeine listing.

$$\% \text{ of error} = \frac{\text{experimental value} - \text{theoretical value}}{\text{theoretical value}} \times 100$$

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

Name _____

DATA TABLE 3

Soft Drink	Caffeine Concentration From Calibration Curve (mg/mL)	Companies' Caffeine Concentration Value (mg/mL)	% Error

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LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

TEACHERS' GUIDE DETERMINING CAFFEINE CONCENTRATIONS IN SOFT DRINKS

CLASSROOM USAGE

This investigation is intended for either a Chemistry 1 or 2 curriculum.

TIME

This lab has two options that determine the number of days that would be required. If option one is used you will need to run the standards one day then the soft drink samples the next. This option has the benefit of more HPLC usage and having students do dilution work with pipets. Option two does not require the running of standards, which probably saves one day. Students begin by working on their photocopied standards and preparation of their soft drinks for injection. Once they have this done they can go to the HPLC for their run. Option one with pre-lab and post-lab would probably take 3 - 4 days. Option 2 would be 2-3 days.

NOTES

Here are some of the companies listed caffeine concentrations.

Tab =	.14 mg/ml
Coke Classic =	.14 mg/ml
Coke II =	.14 mg/ml
Diet Coke =	.14 mg/ml
Pepsi =	.11 mg/ml
Mnt. Dew =	.15 mg/ml
Mello Yello =	.15 mg/ml
Jolt =	.20 mg/ml

(Any others used can be found by calling the manufacturer.)

2. Emphasize to the student that they must dilute the soft drink sample 1:1. The soft drink must be a 50% solution. Pure soft drink will clog the column. This avoids gumming up the column and injection port with sugar. Also, since this 1:1 dilution has been done they have to multiply their caffeine value they get from a calibration curve by 2.
3. After the last students has made their run, or if the pressure exceeds 4000 psi, inject 50 microliters of filtered distilled water through the HPLC to dissolve any excess sugar.

LIQUID CHROMATOGRAPHY

PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

4. All the DATA TABLES are included for your convenience. You may want to pull out the ones which you don't intend to use.

ASSESSMENT

CHECKLIST

-exhibits on task behavior	1	2	3	(Circle One)
-is contributing with lab partner	1	2	3	
-makes careful measurements	1	2	3	
-made accurate caffeine standard	1	2	3	
-used good syringe technique	1	2	3	
-shows evidence of understanding procedure		1	2	3
-cleaned up work area	1	2	3	
-made accurate calculations for class data	1	2	3	
-made appropriate observations	1	2	3	
-wore goggles when working at HPLC	1	2	3	
-acceptable percentage of error	1	2	3	

ACTIVITIES FOR PORTFOLIO

1. Vee diagram
2. pre-lab essays
3. tape procedure pages into portfolio
4. complete all data tables with class data
5. determine the % of error for their soft drink concentration