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Expt #2: High Performance Liquid Chromatographic Determination of Caffeine

Date Began: 2/10/00 Jed Cluson Date Completed: 2/10/00 J.C.

Unknown # Coke + Surge

NOTICE
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Purpose: To determine the concentration of caffeine in sodas using High Performance Liquid Chromatography (HPLC)

Theory: HPLC is a form of column chromatography in which a stationary phase is held in a narrow tube while a mobile phase is forced through under pressure. Column chromatography comes in two forms, normal and reverse phase. In normal phase a polar stationary phase retains polar compounds while the non-polar mobile phase elutes like compounds first. In our lab we will use a reverse phase - a non-polar stationary phase of a alkylated-silica column and a polar alcohol/water mobile phase.

The separation in HPLC rely heavily on plate theory of chromatography. This is mainly used to describe the efficiency of the column. The length of the column and the number of plates are related by the equation $N = L/H$ where H is plate height.

Much more

-20

1. Skoog, D.A., Holler, F.J., Nieman, T.A. Principles of Instrumental Analysis 5th Ed. Philadelphia: Harcourt Brace. 1998. 688.

The longer the column and smaller the plate height, the more efficient the column is. These plates are actually theoretical layers within the column, at each the solute equilibrates between the mobile and stationary phase.

HPLC is the most popular analytical separation technique. HPLC reduces band broadening by reducing particle size and increasing column length. This band broadening is also related to the Van Deemter Equation which describes plate height -

$H = A + B/u + Cu$ where each term is related to a cause of band broading: flow paths, diffusion and mass transfer.

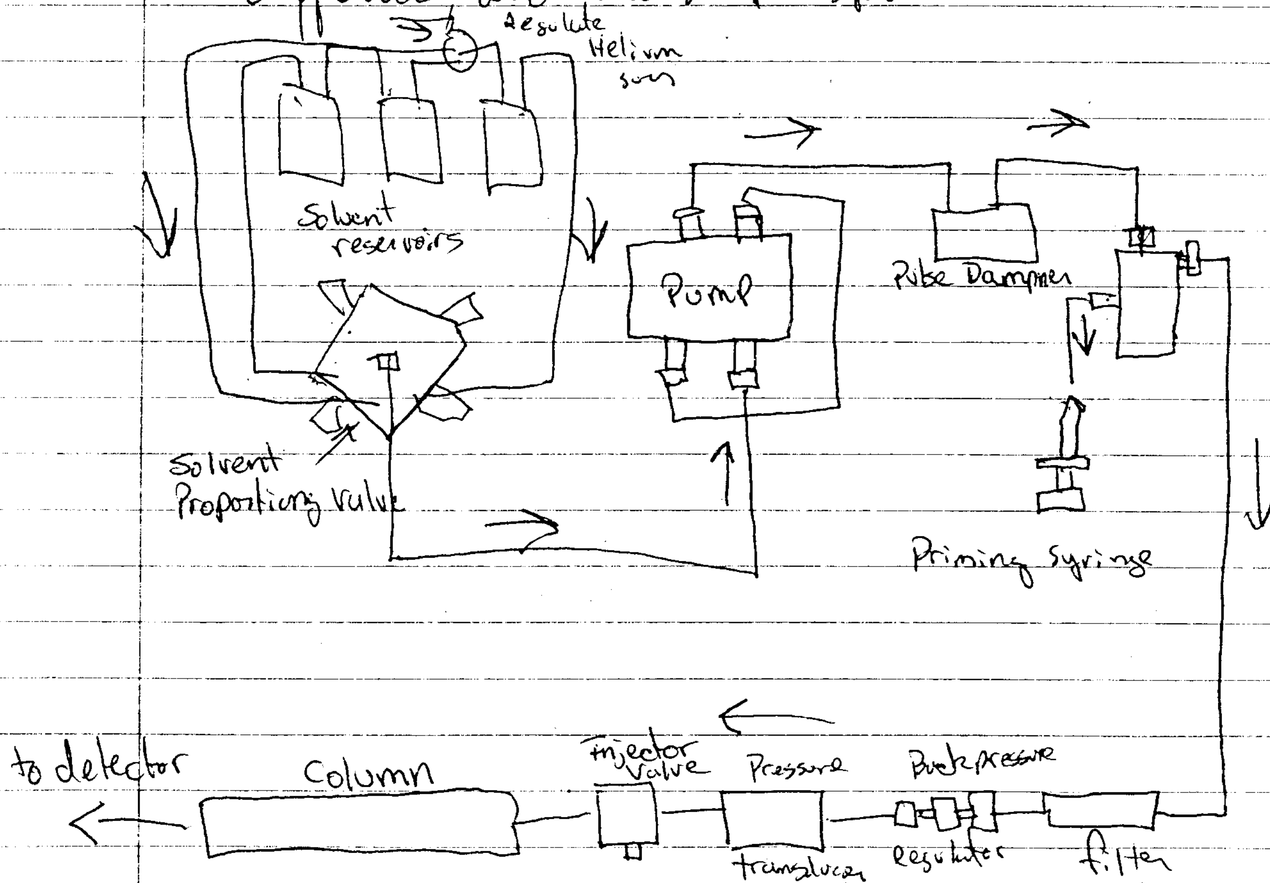


Fig 1. HPLC Apparatus [2]

2. Skoog et. al. p 729

No cookbook procedures

Instrumentation:
A typical Perkin-Elmer HPLC setup is given in Fig 1.

1.) Pump - HPLC's need pump pressures of up to 6000 psi with flowrates ranging from 0.1 to 10 mL/min to provide optimal separations

2.) Filter - removes any foreign particles to assure purity of solvent before reaching column.

3) Column - This is where the separation takes place. As noted before, the longer the column and smaller the packing particles, the better the separations. Columns are either normal phase or reverse phase. The column in this lab is reverse phase. Most common columns are 25cm in length and packed with 5 μm particles, these columns contain 40,000 - 60,000 plates/meter.[3]

4) Injector valve - Samples are loaded here and are picked up by the mobile phase as it leaves the pressure regulator and reaches the column.

5) Solvent reservoirs - Mobile phase is stored here and fed to the pump through the proportioning valve

3. Skoog et. al. p 732

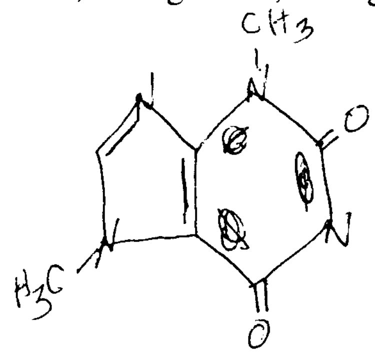
MSDS: www.physchem.ox.ac.uk

Safety data for caffeine

Stability

Stable. Incompatible with strong acids, strong bases, strong oxidizing agents.

Toxicology



Harmful if swallowed.

Toxicity data
ORL-RAT LD50 192 mg/kg.
IPR-RAT LD50 260 mg/kg.
IV-RAT 105 mg/kg.

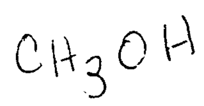
Transport information

UN Major hazard class
Packing group

Personal protection

Safety glasses.

Methyl Alcohol



›tability

Stable. Incompatible with acids, acid chlorides, acid anhydrides, oxidizing agents. reducing ager alkali metals. Protect from moisture. Highly flammable.

›oxicology

Toxic by inhalation, ingestion or skin absorption. **May be a reproductive hazard. Ingestion r be fatal. Exposure may cause eye, kidney, heart and liver damage**. Irritant.

Procedure:

1.) Preparation of Standards

Weigh out five quantities of caffeine in amounts of 2.5 mg; 5.0 mg; 7.5 mg; 10.0 mg; and 12.5 mg into five clean, dry 100 ml volumetric flasks. Dilute each to the mark with the HPLC solvent. Solvent should be 20% methanol / 80% water adjusted to pH of 3.50 with glacial acetic acid. Shake the solutions to ensure mixing and degas for 5 min. prior to injection.

Turn the pumps detector on. Set for a flow rate of 1.5 mL / min and detector sensitivity for 0.08 AUFS (Absorbance unit full scale). Turn the recorder on at slow speed and allow the mobile phase (solvent) to pass through for 5 to 10 min. Record detector response to make sure nothing remains on column.

Using a syringe remove 25 uL from the least concentrated standards and inject each into the HPLC in the LOAD position. The port only delivers 20 uL but 5 uL excess is needed. Turn valve from LOAD to INJECT and push MARKER on detector. This marks when the sample was loaded. Allow the peak due to caffeine to be recorded. Follow the same procedure for the least concentrated sample until you have 3 chromatograms. Repeat, increasing with concentration until you have 15 chromatograms.

2.) Caffeine in Beverages

Pour 10 to 15 ml of each beverage into separate, clean dry beakers. Pour the sample from one beaker into another clean, dry beaker. Pour back and forth until the sample stops bubbling ("flat").

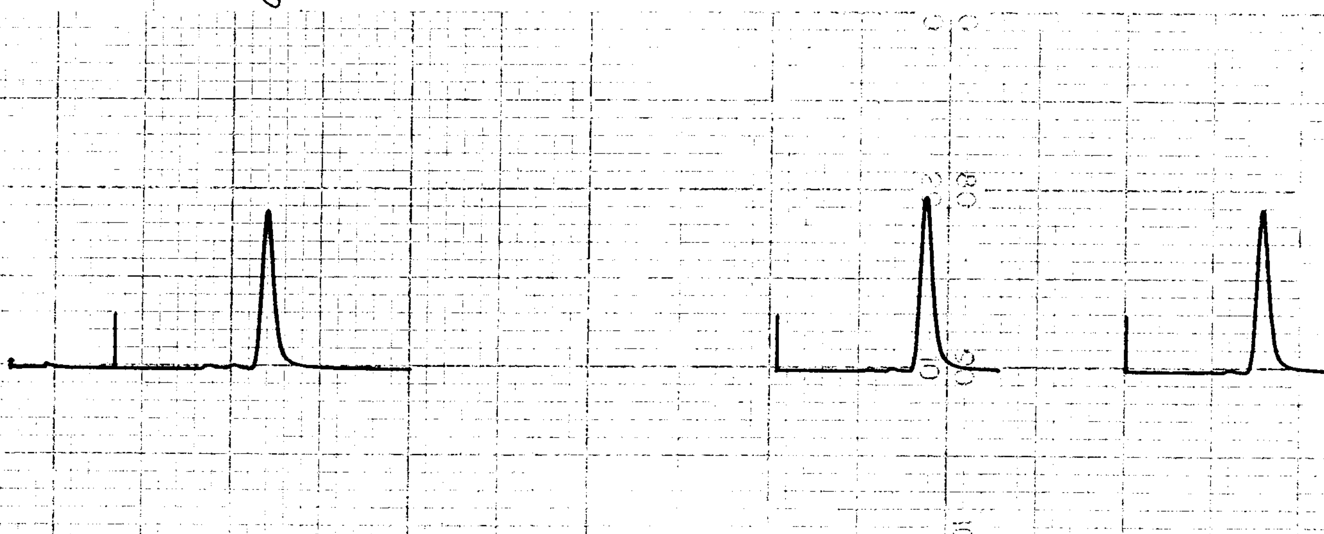
The samples should then be sufficiently decarbonated. Pipet 8 mL of each into separate 25 mL volumetric flasks. Dilute to the mark with the mobile phase solvent used in Part 7. Load the samples onto the HPLC and record 3 chromatographs for each sample. After the last sample is run flush the instrument with an 80% water/20% methanol solution that is not acidic.

Data & Results: [illegible]

Table this information better

~~Det~~ Recorder: 0.2 $\frac{mm}{sec}$ 50 mV

2.5 mg standard — 0.025 mg/mL

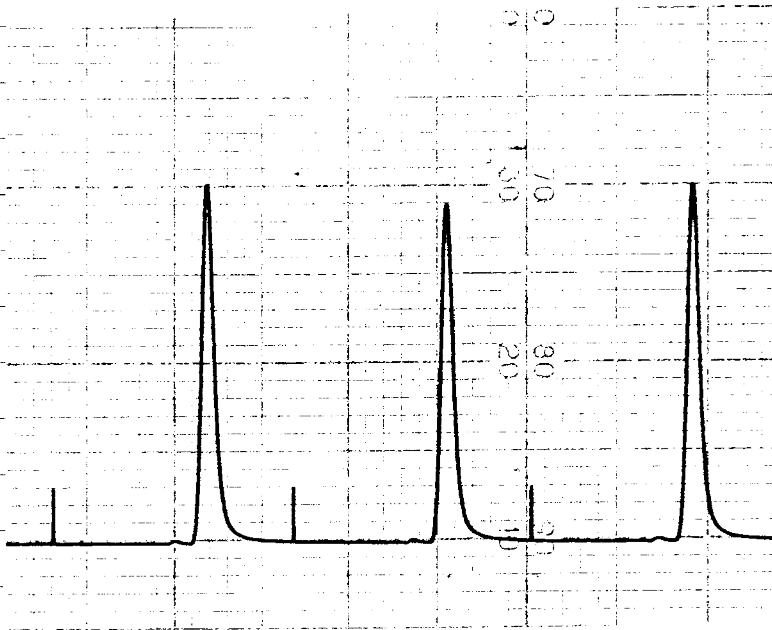


t_{r_1} = 1.6 cm t_{r_2} = 1.5 cm t_{r_3} = 1.5 cm

*$A_1 = 0.475\ cm^2$ $A_2 = 0.500\ cm^2$ $A_3 = 0.475\ cm^2$

* calculated using $A = \frac{1}{2} b_{peak} h_{peak}$ → triangular approximation

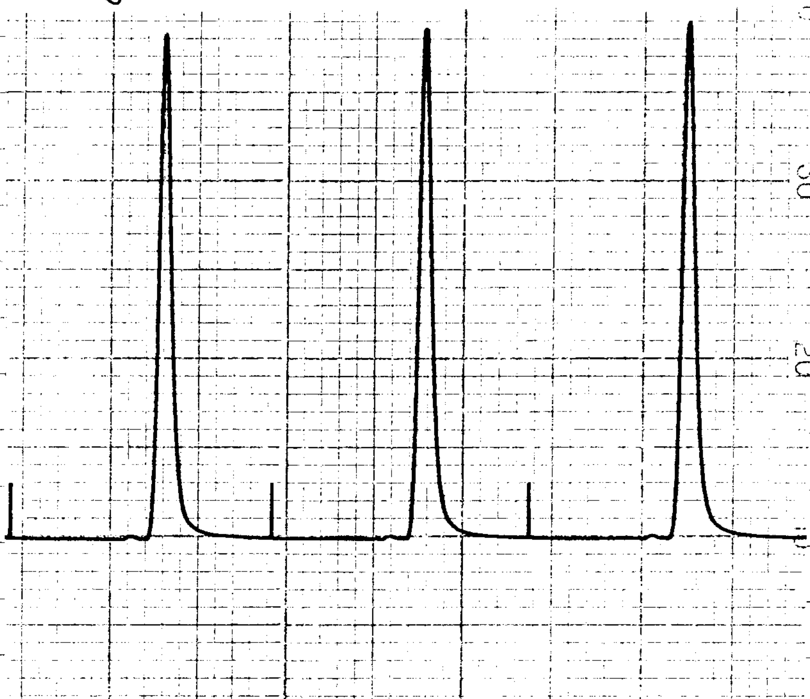
5.0 mg Standard 0.050 mg/ml



t_{r_1} = 1.6cm t_{r_2} = 1.6cm
t_{r_3} = 1.6cm

A_1 = 1.23cm^2
A_2 = 1.17cm^2
A_3 = 1.23cm^2 → Avg + std

7.5 mg Standard 0.075 mg/ml

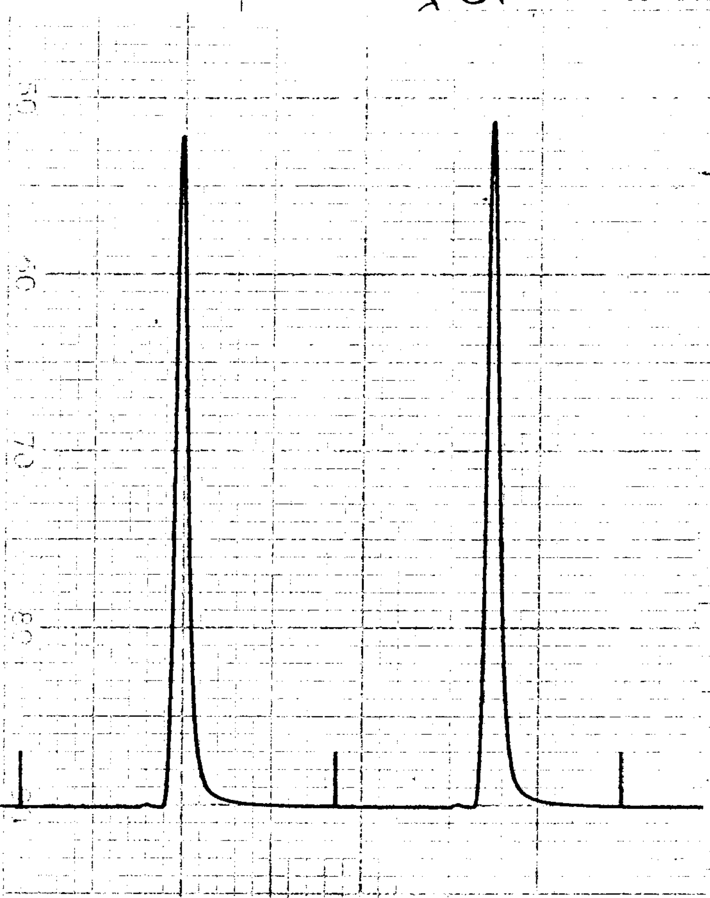


t_{r_1} = 1.5cm t_{r_2} = 1.5cm
t_{r_3} = 1.6cm

A_1 = 1.74cm^2
A_2 = 1.74cm^2
A_3 = 1.77cm^2 → Avg + std

2·8

10.0 mg Standard 0.100 mg/ml

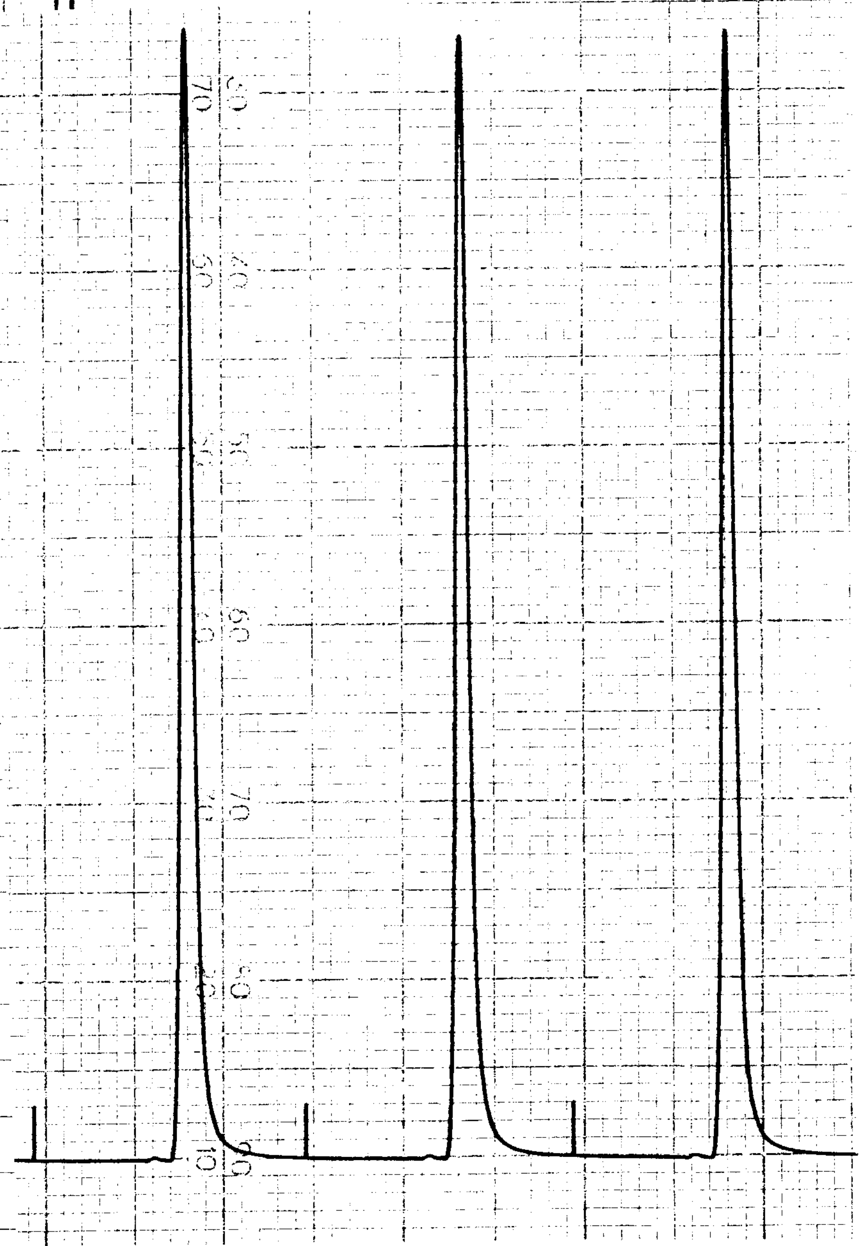


t_{r_1} = 1.6 t_{r_2} = 1.5 t_{r_3} = 1.5

A_1 = 2.31 cm^2
A_2 = 2.34 cm^2
A_3 = 2.55 cm^2

Avg Std

12.5 mg Standard 0.125 mg/ml



t_{r_1} = 1.6
t_{r_2} = 1.5 t_{r_3} = 1.5

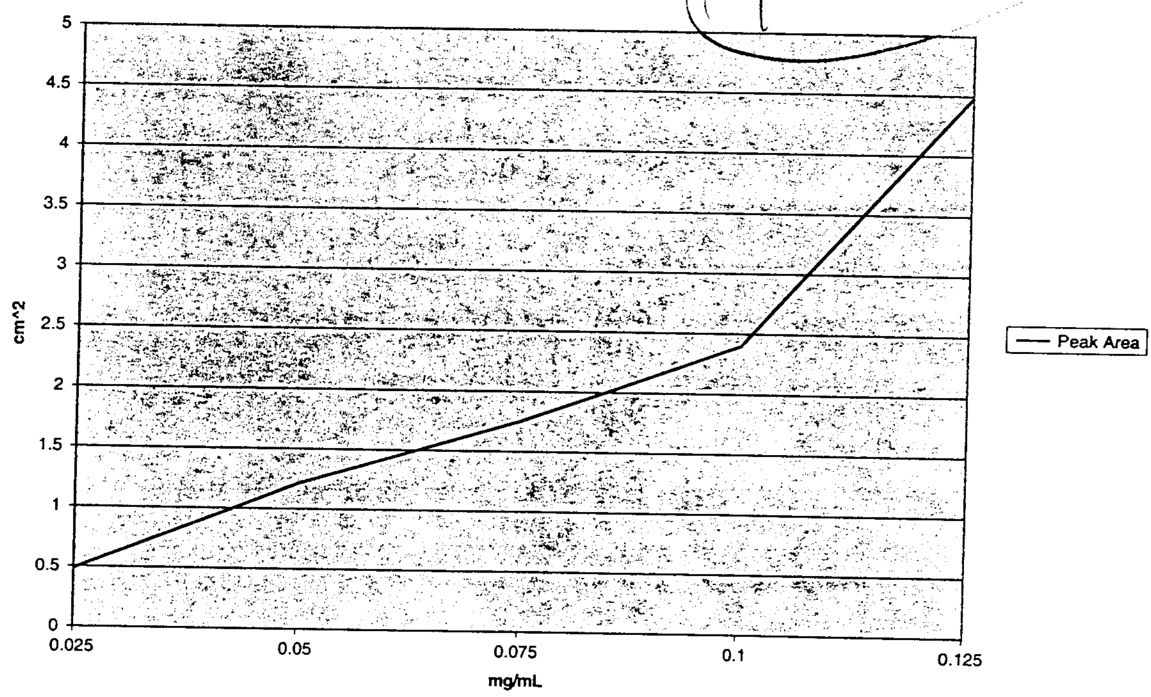
A_1 = 4.52 cm^2
A_2 = 4.45 cm^2
A_3 = 4.48 cm^2

Avg Std

Summary of Standard Results

OK

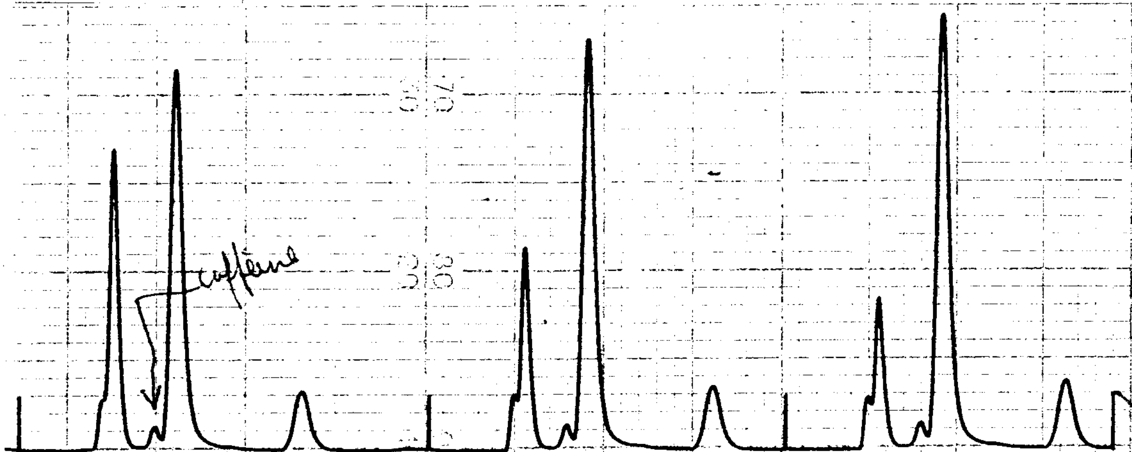
Concentration	Retention Time	Area
0.025 mg/ml	1.53 ± 0.06 cm	0.483 ± 0.014 cm^2
0.050 mg/ml	1.60 ± 0.0 cm	1.21 ± 0.03 cm^2
0.075 mg/ml	~~1.23~~ 1.53 ± 0.036 cm	1.75 ± 0.020 cm^2
0.100 mg/ml	1.53 ± 0.06 cm	2.40 ± 0.13 cm^2
0.125 mg/ml	1.53 ± 0.06 cm	4.48 ± 0.04 cm^2



$$\text{Concentration} = \frac{\text{Peak Area} + 0.6906}{36.736}$$

$R^2 = 0.90915$ Std. Error = 0.5300

Surge ~~Coke~~ Sample

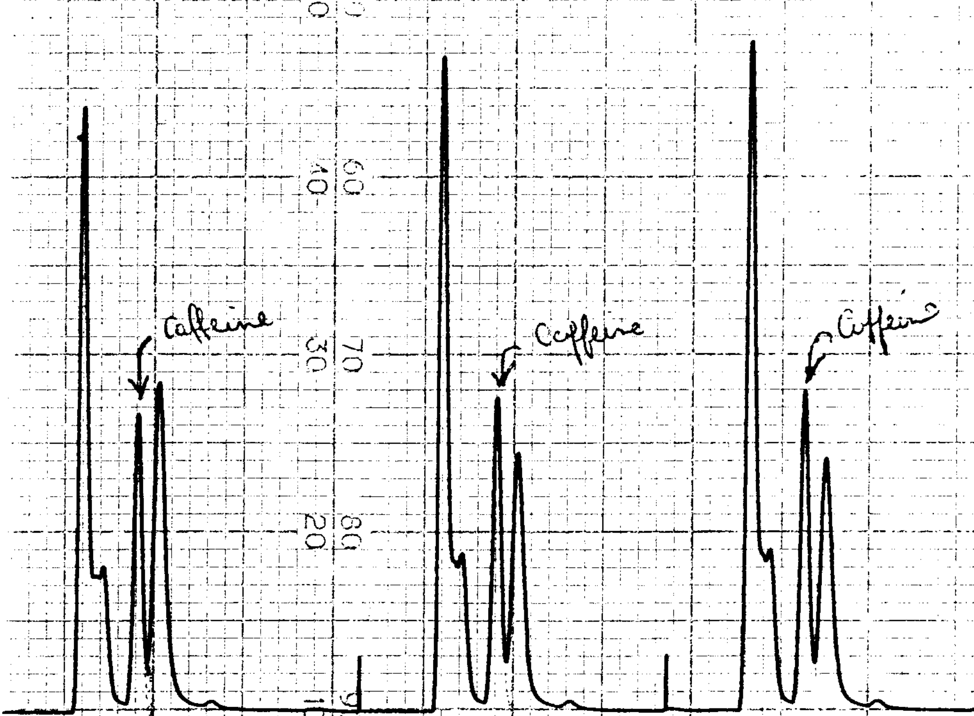


$t_{r_1} = 1.6$ cm
$t_{r_2} = 1.5$ cm
$t_{r_3} = 1.5$ cm
$A_1 = 0.045$ cm^2
$A_2 = 0.045$ cm^2
$A_3 = 0.070$ cm^2

$\bar{t}_r = 1.5$ cm $\bar{A} = 0.053 \pm 0.014$ cm^2

Concentration = $0.0202 \frac{mg}{mL}$ in sample $0.0633 \frac{mg}{mL}$

Coke ~~Surge~~ Sample



$t_{r_1} = 1.5$ cm $A_1 = 0.680$ cm^2
$t_{r_2} = 1.5$ cm $A_2 = 0.700$ cm^2
$t_{r_3} = 1.5$ cm $A_3 = 0.700$ cm^2
$\bar{t}_r = 1.5$ cm $\bar{A} = 0.693 \pm 0.012$ cm

Analyzed sample
Concentration = $0.0377 \frac{mg}{mL}$
So, 0.9425 mg in 25 ml
in 8 mL sample
$\frac{0.9425 \text{ mg}}{8 \text{ mL}} = 0.118 \frac{mg}{mL}$

Conclusion:

Standard caffeine solution were run on HPLC and produced fairly uniform results. The average retention time of the caffeine peak was about 1.53 cm and this was used to identify the caffeine peak in the soft drink samples. The peak areas were ~~estimated~~ calculated by treating the peak as a triangle and measuring the base and height of the peak. These areas were used to plot a calibration curve of concentration vs peak area. The graph is nearly linear though a jump does occur between 0.1 mg/ml and 0.125 mg/ml. This could be due to human error in the formation of the 0.125 mg/ml solution. A least-square analysis gives an equation for the concentration based on peak area and an R^2 of 0.909 shows pretty good fit to the data.

Using a retention time of 1.53 cm, the caffeine peaks on the soft drink chromatograms were identified. The areas of these peaks were calculated in much the same way as the standards. These values were plugged into the calibration equation to give the concentrations of the dilute, 25mL solutions. A calculation was performed to find the concentration of caffeine in the original 8mL. The results show that Coke has nearly twice as much caffeine as Surge.

math & probs!
Explain more about the linearity of the graph. Should run one or more of the std's?
More

1. You could run standards on UV/Vis or GC, run the samples and identify the peaks and concentration based on the standards

2. The two methods used by the articles involve FTIR[1] and electrocapillary phosphoresis[2] the FTIR method involves more complicated machinery but reduces the solvent needs of HPLC to less than 30 mL of $CHCl_3$. The capillary electrophosphoresis allows for simultaneous detection of aspartame, benzoic acid, and caffeine. This could also be done with HPLC, but standards of each would have to be run.

3. Not in the standards, but in the samples yes. I'm not sure if these were interferences or just other components of the soft drinks (sugar, dyes, etc) Interferences should get worse at 254 nm. More energy in the photons should strengthen interference

4. normal phase - benzene, anthracene, napthalene
reverse phase - napthalene, benzene, anthracean

[1] Bouhsain, Z. Garrigues, J.M., and Garrigues S. Vibrational Spectroscopy. 1999. 21, 143-150
2. Walker, JC, Zaugg, SE, and Walker, EB. Journal of Chromatography A. 1997. 781, 481-485.