How Much Caffeine, Aspartame and Sodium Benzoate are in Your Soda?
Grade/Subject: Middle School Science

Purpose
The purpose of this activity is to separate the components of a mixture.

Instructional Objective
Students will measure and compare the relative amounts of caffeine, aspartame and sodium benzoate found in a soda by using a high performance liquid chromatograph (HPLC).

Science Standards
6.2.9 Compare consumer products, such as generic and brand-name products, and consider reasonable personal trade-offs among them on the basis of features, performance, durability, and costs.
7.2.6 Read analog and digital meters on instruments used to make direct measurements of length, volume, weight, elapsed time, rates, or temperatures, and choose appropriate units.
8.2.2 Determine in what unit, such as seconds, meters, grams, etc., an answer should be expressed based on the units of the inputs to the calculation.
8.2.4 Use technological devices, such as calculators and computers, to perform calculations.

Interstate New Teacher Assessment and Support Consortium
Principle 1: CONTENT
The teacher of science understands the central ideas, tools of inquiry, applications, structure of science and of the science disciplines he or she teaches and can create learning activities that make these aspects of content meaningful to students.

Principle 7: CURRICULUM DECISIONS
The teacher of science plans instruction based upon knowledge of subject matter, students, the community, and curriculum goals.

Materials
HPLC
HPLC buffer
Beaker
Syringe, 100 iL
Graduated cylinder, 10 mL
Plastic flask
Various brands and types of regular and diet soda (Coke, Diet. Dr. Pepper, etc.)
Home made soda pop

Safety Considerations
The HPLC buffer is made of methanol, acetic acid and deionized water. Methanol and acetic acid are flammable liquids and vapors. Both cause eye and skin irritation. Has caused adverse reproductive and fetal effects in animals. Harmful if inhaled. May be fatal or cause blindness if swallowed. Wash thoroughly after handling either chemical. Keep away from heat, sparks, and flame. Keep away from sources of ignition. Do not store in direct sunlight. Store in a tightly closed container. Keep methanol from contact with oxidizing materials and acetic acid above its freezing point (62° F). Store both chemicals in a cool, dry, well-ventilated area away from incompatible substances.
Anticipatory Set
Making your own soda pop (See attachment)
NOTE – Home made soda requires 4-6 days of fermentation. Therefore, the teachers should begin a batch at least one week before lab is to begin.

Instructional Input
- **Input**: The teacher can use lecture, instrumentation video clips/pictures (See Resources), etc. to introduce basic chromatography principles to the students. The teacher can run various pop samples, and make the chromatographs available for the students to study.
- **Modeling**: Once the material has been presented, the teacher can then make analogies between the anticipatory set and the actual lab to show students examples of what is expected as an end product of their work. Once the home made pop is finished being made, the teacher can run a sample of that pop through the HPLC, so the students can compare their pop to the store bought brands.
- **Check for Understanding**: The teacher can visually inspect each student (or lab group) as they come up to the HPLC to inject their samples to make sure that they are doing it in the correct method. While the sample is running, the teacher can ask questions about how the HPLC works.

Guided Practice
The teacher can show sample HPLC graphs to the class and ask the class about the importance of each part of the graph (i.e. air peak, caffeine peak, retention time, peak area). Running caffeine, aspartame, and sodium benzoate standards is an excellent way to demonstrate the path of a liquid through the HPLC.

Independent Practice
Students will run samples of their soda in the HPLC. Using the HPLC graphs, the teacher can have students match each part of the graph to the corresponding label. Students should be able to identify and label each part of the graph and compare the peaks for caffeine, aspartame and sodium benzoate.

Closure
Briefly recap the vocabulary associated with the HPLC graph and have students identify each word to its corresponding part on a picture of a HPLC graph (possibly pre-printed on a transparency).

Assessment
Teachers can assess students through worksheets, quizzes or tests. The following is a list of possible questions that may be asked about the lab:
1. How did your pop caffeine value compare to the standards given by your teacher? Aspartame? Sodium benzoate? What could have caused discrepancies between these your values and the can’s values?
2. From the data, which ingredients (caffeine, aspartame, sodium benzoate) were typically found in diet sodas? Regular sodas?
4. List one possible way that this instrument is used in everyday life.
**Real Life Applications**

HPLC has many applications including separation, identification, purification and quantification of various compounds. It is currently used by a variety of fields including pharmaceuticals, biomedical and biochemical research, cosmetics, energy, food and environmental industries. Biotechnology companies use the HPLC to prove to the government that their products are nontoxic, pure and active.

**Teacher Notes**

Sample graphs of each store bought pop should be run the night before, so students have chromatographs to compare to theirs.

Buffer for mobile phase contains 68% methanol, 30% acetic acid and 2% deionized water by volume. The HPLC takes a few minutes to set up. Each sample runs for less than five minutes in the HPLC. **It is extremely important that the students degas their soda sample because any air bubble can clog up the column in the HPLC. It is sometimes difficult to get the pop completely flat – one suggestion might be to let the pop sit open overnight (or a weekend) and possibly in a wide-mouthed container to increase surface area.** The beakers and etc. must also be VERY CLEAN. Dust particles will even clog this column. Be sure your students rinse them with the buffer.

For students who would like to be challenged, the teacher may have them determine the amount of caffeine, aspartame and sodium benzoate that would be found in various brands of coffees and teas.

The following table lists the amount of caffeine that is found per 12 ounce can of soda. This table works well as a follow up or closure activity.

<table>
<thead>
<tr>
<th>Drink Name</th>
<th>Amount of Caffeine (mg)</th>
<th>Drink Name</th>
<th>Amount of Caffeine (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afri-Cola</td>
<td>100.0</td>
<td>Shasta Cola</td>
<td>44.4</td>
</tr>
<tr>
<td>Jolt</td>
<td>71.2</td>
<td>Mr. Pibb</td>
<td>40.8</td>
</tr>
<tr>
<td>Mountain Dew</td>
<td>55.0</td>
<td>Dr. Pepper</td>
<td>39.6</td>
</tr>
<tr>
<td>Diet Mountain Dew</td>
<td>55.0</td>
<td>Pepsi Cola</td>
<td>37.2</td>
</tr>
<tr>
<td>Kick citrus</td>
<td>54.0</td>
<td>RC Cola</td>
<td>36.0</td>
</tr>
<tr>
<td>Mello Yellow</td>
<td>52.8</td>
<td>Diet RC</td>
<td>36.0</td>
</tr>
<tr>
<td>Surge</td>
<td>51.0</td>
<td>Diet Pepsi</td>
<td>35.4</td>
</tr>
<tr>
<td>Tab</td>
<td>46.8</td>
<td>Canada Dry</td>
<td>30.0</td>
</tr>
<tr>
<td>Coca-Cola</td>
<td>45.6</td>
<td>Diet Rite</td>
<td>6.0</td>
</tr>
<tr>
<td>Diet Coke</td>
<td>45.6</td>
<td>Diet Canada Dry</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Math standards can also be covered, through having students find mean, median, and mode data. Graphs can also be easily produced from the student data.

Downtime during this activity should be filled by having students work on charts, graphs, computer research, and vocabulary.

**Resources**

Chasteen, T. G., Sam Houston State University, Department of Chemistry. Chemistry-Based QuickTime, Shockwave Flash, GIF Animations and Streaming Audio. Retrieved June 13, 2003 from [http://www.shsu.edu/%7EChasten_tgc/sounds/sound.html](http://www.shsu.edu/%7EChasten_tgc/sounds/sound.html) (instrumentation video clips)
References
How Much Caffeine, Aspartame and Sodium Benzoate are in Your Soda?

Introduction

In this experiment, you will use the HPLC to test various soda pops for caffeine content. We will be able to determine how much caffeine is in your soda pop. We will also look for sodium benzoate (a preservative) and aspartame (an artificial sweetener in diet beverages).

You will run a sample of a known standard – caffeine, sodium benzoate, or aspartame – and then run a sample of your soda pop and compare it to the standard. You will compare the relative amount of each chemical to determine which soda pop contains the greatest and least amount of that substance.

Let’s use caffeine as an example.

Run a sample of the caffeine standard, and note how long it takes for the caffeine peak to appear. This is the time it takes for caffeine to run through the HPLC. The higher the peak the more caffeine you have. You will use this principle to compare the levels of caffeine, aspartame, and sodium benzoate in the pop.

Procedure

1. You will need a beaker, a plastic flask, a 10 mL graduated cylinder and soda.
2. Clean ALL containers very well. Do NOT dry them. Rinse ALL glassware with a little buffer.
3. Pour 10-15 mL of your soda pop into a clean plastic flask.
4. Put the top on and shake well. Remove the cap to release pressure.
5. Repeat step 4 until no bubbles appear. VERY IMPORTANT!
6. Measure 7.00 ml of DEFIZZED soda pop into a graduated cylinder.
7. Add buffer to the 10-mL mark. Be careful to measure accurately. Stir the buffer and pop together.
8. Go to the instrument now.
9. Fill the syringe with your soda sample and squirt it out on a paper towel.
10. Fill your syringe with 100 iL of your sample.
11. Follow the injection instructions. (see below)
12. When a peak comes out on the chart paper, determine which chemical is responsible.
13. Repeat steps 9-12 for your each pop sample you test.
14. Tear off the chart paper with your result. Label each peak.
15. Clean your syringe with lots of buffer after you are done.

Closing Question:
What chemical appeared the most? Why do you think that it did?