WHAT IS IN YOUR PAIN RELIEVER

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INTRODUCTION

Headache, sore muscles, arthritis pain... How do you spell relief? Pain serves the useful function of alerting you when some component of a physiological system has gone awry. Ideally, the pain can be alleviated by removing the underlying cause. In many cases, however, the stimulus of pain is either not easily defined or is not readily removed; therefore, it is necessary to treat pain as a symptom.

As a result, Americans spend millions of dollars annually on NON-NARCOTIC ANALGESICS sold as over-the-counter pain relievers. Pharmaceutical companies go to great lengths to convince you that Anacin® works twice as fast or that you can "Nupe it with Nuprin®". But the bottom line is that there are only three non-narcotic pain relievers which are considered safe enough to be sold over-the-counter.

The oldest of these is aspirin, which has the chemical name acetylsalicylic acid. Salicylates were discovered following the extraction of a naturally occurring substance, glycoside salicin, from willow bark. The original compounds were found to have medicinal value but also some side effects. Further investigations led to aspirin, an ester derivative of salicylic acid, which was first marketed commercially by the Bayer Company in Germany in 1899.

Acetaminophen became available around 1950. While acetaminophen relieves pain and reduces fever, it does not relieve stiffness, redness and swelling of arthritis as aspirin does.

Ibuprofen was first used predominantly for its anti-inflammatory activity. It was first released in 1984 for over-the-counter use and has now replaced some of the market share of aspirin-based compounds.

Caffeine is not a pain reliever, although it is sometimes found in these products. Caffeine acts by stimulating the central nervous system, and when present in some of these products may provide additional relief of headache pain or faster relief.

This is a qualitative experiment and in it we will determine which of the four compounds (aspirin, acetaminophen, ibuprofen and/or caffeine) are present in a given relief product. In order to identify the compounds, we will need to separate them. To do this we will utilize a High Performance (Pressure) Liquid Chromatograph or HPLC.

Chromatography consists of two phases: the mobile phase and the stationary phase. In our experiment our mobile phase is the solution containing 30% methanol, 2% acetic acid, and 68% distilled water. The stationary phase contained in the column consists of silica beads coated with

"C-18" chains. In other words, the surface of our stationary phase consists of hydrocarbon chains containing 18 carbons. For example:

(silica bead) $-CH_2-CH_2-CH_2-CH_2-CH_2-(CH_2)_{10}-CH_2-CH_2-CH_3$ ←surface

One important property of our phases is their relative polarity. Our mobile phase is much more **polar** than our stationary phase. Why do we care about the polarities? Let's imagine that we have two different type of molecules at the start of the column, molecule A and molecule B. Now if A is more polar than B, A will be attracted to the mobile phase than B. Since A is more attracted to the mobile phase, it will be moving with the mobile phase a larger percentage of the time than B will since B is more attracted to the stationary phase. We can see then that A will move through the column faster than B does. In other words, A would have a lower **retention time**. **Retention time** is the amount of time that a compound takes to pass through a column. By marking our chromatogram, we can determine the retention time by determining the time from the **solvent front** to the middle of our retained peak. The **solvent front** is the peak that occurs when the non-retained part of the injection first passes through the detector.

After passing through the column, the molecules in solution pass through a detector. Our detector measures how much the solution absorbs ultraviolet radiation with a wavelength of 254 nm. Solutions that contain greater concentrations of molecules that absorb at this wavelength will show larger "peaks". Unless we make a standard curve, though, we cannot look at our chromatogram and compare concentrations of different peaks by comparing the sizes of different peaks. The reason we cannot is that different compounds have different molar absorptivities (or a relative ability to absorb radiation) at 254 nm. In other words, a one molar solution of aspirin will have a different sized peak on a chromatogram than a one molar solution of acetaminophen.

One other problem we can run into is that some compounds may have similar retention times. For instance, in our lab, ibuprofen and aspirin have essentially the same retention time. Unless we run further tests on our unknown samples, we will only be able to verify that our unknowns contain either aspirin or ibuprofen. For this reason, your teacher may opt to only test for acetaminophen, aspirin, and caffeine.

PURPOSE

The purpose of this laboratory is to introduce the HPLC as a tool for identifying and separating compounds by measuring different retention times.

MATERIALS

HPLC equipment

Mobile phase: [Provided by Chemobile]
30% methanol (300 mL methanol, 20 mL acetic acid, 680 mL deionized water - used for experiment)
75% methanol (750 mL methanol, 250 mL deionized water - used for storage)
Known standards
Pain relievers to serve as unknowns

PROCEDURE

Preparation and Injection of Standards

1. Prepare qualitative standards for pure caffeine, acetaminophen, and aspirin. The solutions should contain roughly 200-300 mg/100 mL distilled water.

2. Refer to "**HPLC Instructions For Operation**". NOTE: Remember to filter all samples before injection!

3. Inject your standards one at a time at AUFS* value 1.0.
   *AUFS = Absorbance units full scale. Therefore, 1.0 AUFS means that an absorbance reading of 1.0 would register full scale on the chart recorder.

4. Record the following information on the chart paper:
   Mark the point of injection!
   AUFS value
   Identity of sample
   Chart speed

5. Allow each sample time to elute before injecting another sample.

6. Remove paper from chart recorder.

7. Determine the **retention time** of each compound. (This is the time from the **solvent front** until the middle of the peak.) For instance, if the chart speed is 2.5 cm/min and the distance between **solvent front** and the middle of the peak is 4.5 cm, then the **retention time** is 1.8 min. The solvent front is usually the first peak. It will usually go "up" and then go "down" or below zero so we get a peak that looks like one cycle of a sine wave.

Preparation and Injection of Unknown Sample

1. Crush your sample of cold medicine using a mortar and pestle, and dissolve it in distilled water.

   NOTE: Since we are doing a qualitative analysis, the sample does not need to be entirely dissolved. It is essential, though, that you FILTER each sample before injection into the HPLC.

2. Inject the samples according to "**HPLC Instructions For Operation**". If the peaks are too small, set the AUFS Range to one value lower and inject the sample again.

3. After getting an acceptable chromatogram, remove the paper from the chart recorder and calculate retention time as you did for the standards.

Identification of Compounds

Comparing **retention times** should give a good estimate of the identity of the compound(s) in the pain reliever. To "confirm" the identification, you could "spike" the unknown sample with known standards.

For instance, if you want to confirm that you have a caffeine peak, combine a small amount of caffeine solution with a small amount of your sample solution. Inject the filtered sample and compare with the first injection of your sample. If you are correct, the peak which you identified should be noticeably larger.

Remember, in the case of the aspirin and the ibuprofen which have similar retention times, we can only confirm that we have aspirin and/or ibuprofen. Final confirmation on any of the unknowns can be done by checking the label on the product.

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TEACHERS' GUIDE
WHAT'S IN YOUR PAIN RELIEVER?

CLASSROOM USAGE

This laboratory could be used for first or second year chemistry students.

CURRICULUM INTEGRATION

This laboratory exemplifies physical separations and could be utilized in an early unit involved with separations and/or polarity. The students should have some understanding of polarity and some understanding of solutions.

PREPARATION

See **HPLC Instructions For Operation**.

TIME

TEACHER: Getting the HPLC up and running will take 1 hour.
STUDENTS: Preparing samples will take approximately 10 minutes per sample.
Each run on the HPLC will last about 5 minutes.

SAFETY AND DISPOSAL

All solutions can be washed down the drain.

NOTE ON PROCEDURE

Due to the lack of a pure ibuprofen standard and a good method for including ibuprofen in a successful separation, the lab has been designed to omit ibuprofen. Teachers may want to experiment with ibuprofen as a classroom project.

VARIATIONS

This lab is an example of **Reversed-Phase** Chromatography. (The stationary phase is less polar than the mobile phase.) For a good contrast, you could have the students perform a separation of Grape Kool-Aid® on a Sep-Pak (or any C18 separation cartridge) and also on chromatography paper with water as the mobile phase. The Sep-Pak is **reversed-phase** chromatography, and the paper is **normal-phased** (the water/paper stationary phase is more polar than the water mobile phase). The order of elution is different for the two Kool-Aid separations.