

Thin Layer Chromatography

Purpose: To use thin layer chromatography (TLC) to determine the probable identity of the major active ingredients in commercial analgesic preparations.

Reading: *Techniques:* Chromatography (Introduction and TLC)

INTRODUCTION

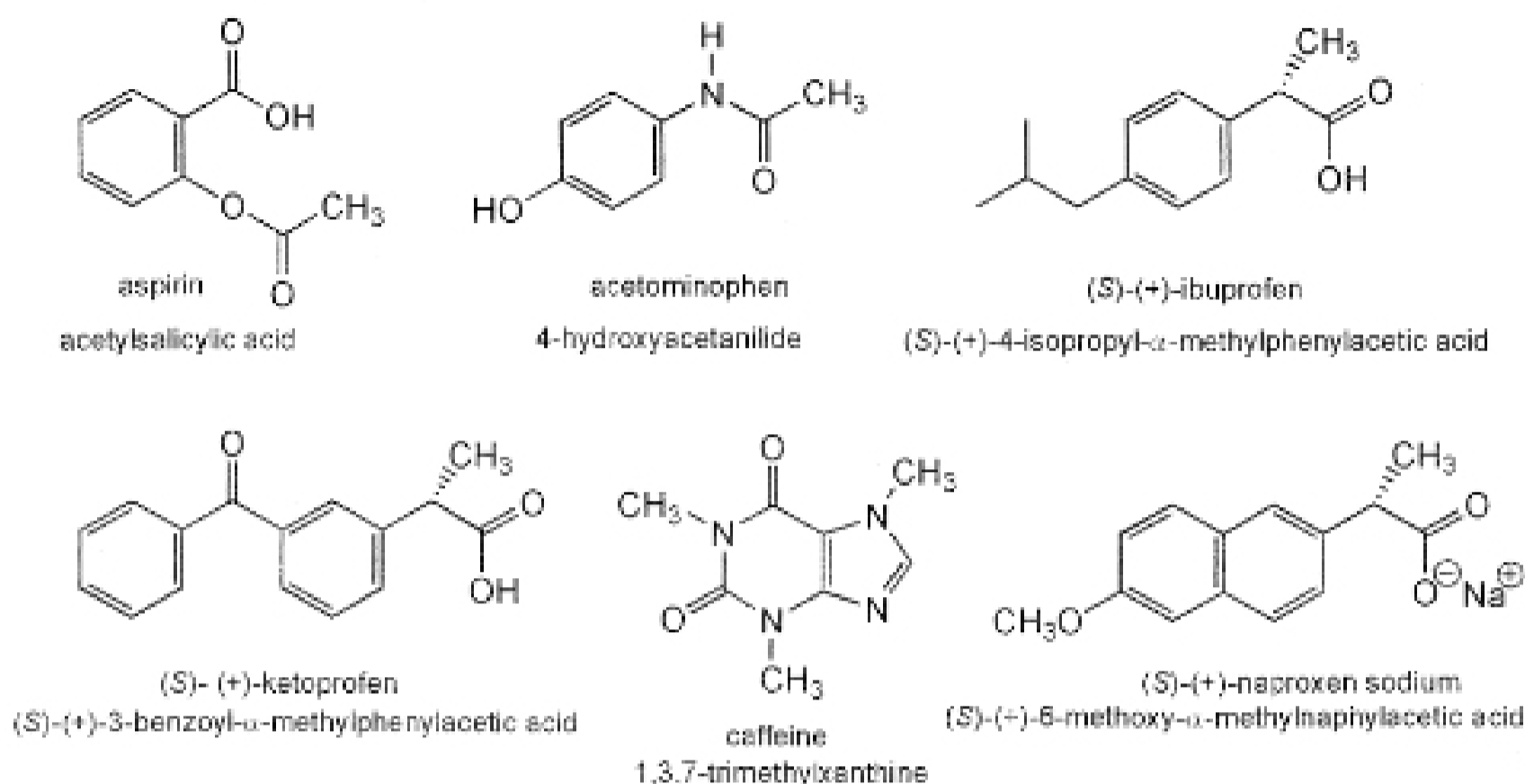
Chromatography is one of the most frequently used organic techniques. Chromatography is valuable not only as a method for isolation and purification, but many types of chromatography are also valuable analytical tools to quantitate amounts of compounds present. In this experiment, you will use normal phase TLC to demonstrate how this technique may be applied in qualitative analysis (what is present, not exactly how much is present).

You will use TLC to analyze common analgesics. Analgesics are pain relievers (anodynes are mild pain relievers). Most analgesic preparations contain either aspirin, acetaminophen, or ibuprofen as the active pain relief agent. In the past, phenacetin was used as an aspirin substitute. Phenacetin is no longer used because it was associated with kidney damage and there is some indication that it might also be a carcinogen. Analgesics may contain ingredients unrelated to the relief of pain. Caffeine is present in several commercial analgesics. It is possible that addition of non-analgesics to these products is more for advertising potential than for increasing the efficacy of the product. In this experiment, you will also observe the importance of the solvent in producing good separation of sample components.

FYL The "Migraine Relief" claim that is now on many analgesics is directly tied to their use of caffeine. Caffeine has many unique biological effects. One of the most interesting is its effect on the vascular system. Caffeine dilates all the blood vessels in the body from the neck down. Curiously, it constricts the blood vessels in the head. Although there is some argument that initial cranial vasoconstriction triggers the migraine attack, there is no argument that vasoconstriction is the only way to stop the attack. As an added bonus, caffeine also cuts the time required for onset of relief roughly in half.

Occasionally, following prolonged study, the FDA will allow a prescription analgesic to be released for OTC (over the counter) use. Most recently ketoprofen (Oravail[®] and Orudis[®]) and naproxen sodium (Anaprox[®] and Aleve[®]) have been released in OTC formulations. Like ibuprofen, ketoprofen is a derivative of 2-propanoic acid. Naproxen is also a derivative of 2-propanoic acid, (*S*)-2-(6-methoxy-2-naphthyl)propanoic acid. Naproxen sodium is the soluble sodium salt of this acid. Of interest, ibuprofen and ketoprofen are sold and used as their racemic mixtures. Naproxen, the prescription drug Naprosyn[®], and naproxen sodium are sold and used as the pure

S enantiomer. The OTC formulations are identical to the prescription drugs albeit at lower concentrations.



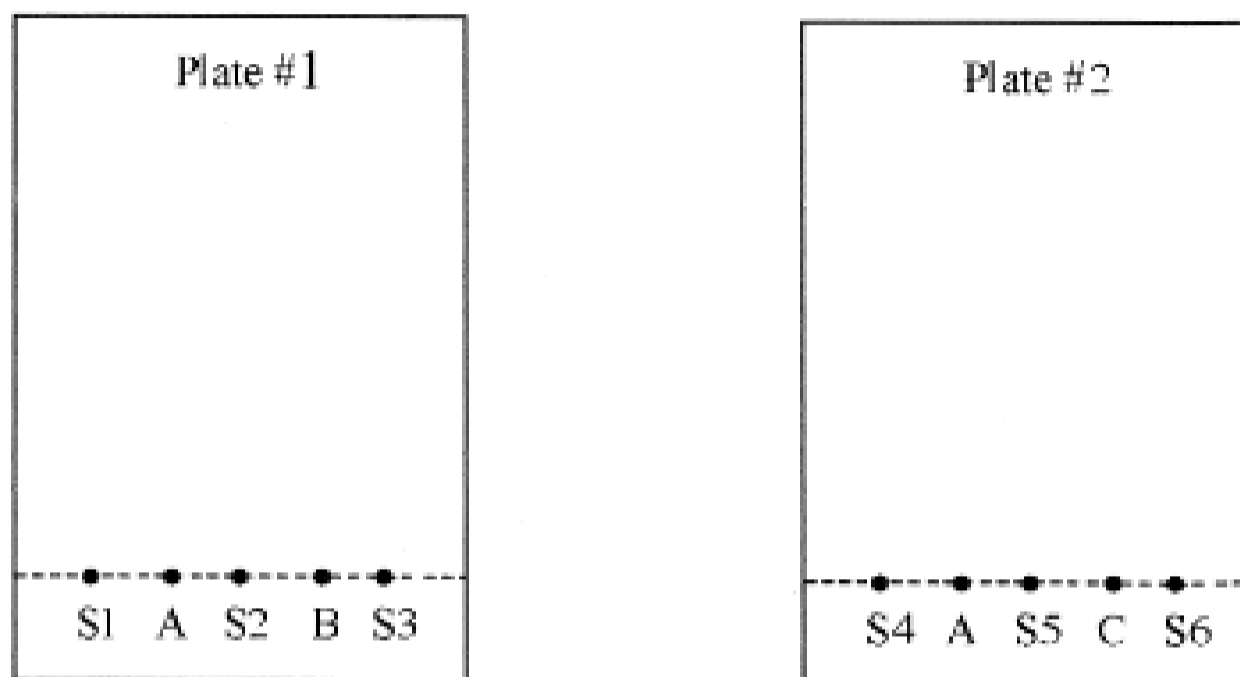
Aspirin is a registered trade name of the Bayer Corporation., because of its name recognition many people use aspirin to describe acetylsalicylic acid (you may notice that many pharmaceutical preparations will use the abbreviation ASA to avoid trademark infringement). While ibuprofen (the generic form of Advil[®] and Motrin[®]) and ketoprofen (the generic form of Orudis[®] and Oravail[®]) are sold as an enantiomeric mixtures, only the *S*-isomer is possesses analgesic activity. Out of curiosity, ibuprofen and ketoprofen conjugate with amino acids in the body (a specific process called glucuronidation) and the glucuronides can (with enzymatic assistance) *R* to *S* interconvert.

EXPERIMENTAL PROCEDURE

TLC of Common Analgesics. You will work in groups of *three* to compare results. Each member of the group will work with a different commercial analgesic sample and a different TLC solvent. Obtain an analgesic tablet, three (3) normal phase TLC sheets, a spotting capillary, and a mortar and pestle from the supply area. Be sure that each student in the group selects a different analgesic tablet. Grind the tablet with four mL of reagent grade methanol and then gravity filter the suspension through a folded 11-cm piece of filter paper in a short stem funnel. Prepare the TLC sheet for spotting, you will be applying five (5) spots to each TLC plate. (The procedure for marking and spotting a TLC plate can be found in the *Techniques: Thin Layer Chromatography*.)

You will be using the same spotting capillary throughout this experiment. You may clean this capillary by wiping the outside with a Kim wipe dampened with acetone. The inside may be washed by pulling up a small amount of methanol and then allowing this to drain onto a paper towel. Doing this several times will be sufficient to clean the spotting capillary. This will allow you to proceed without cross-contaminating the standards. This will also prevent cross-contamination from occurring on your TLC plates.

Solutions of six (6) standards (aspirin, ibuprofen, acetaminophen, ketoprofen, naproxen sodium, and caffeine) are available for your use. Use the templates below to mark your TLC plates prior to spotting. The spots designated as S1 - S6 are the six standard solutions. Spot A is the extract of your analgesic tablet; spots B and C are the extracts of the tablets of the other two students in your group.



Each student in the group will use a different solvent. The three solvents to be used are hexanes, ethyl acetate, and 75% ethyl acetate - 25% hexane. Pour sufficient developing solvent into the TLC jar so there will be about 5 to 6 mm of liquid in the bottom of the jar. Place the spotted TLC sheet in the jar and allow the solvent to ascend the sheet. The jar must be sealed to prevent solvent from evaporating from the surface of the TLC plate. Place the cap on the top of the jar to seal it. Allow the solvent to ascend until it is about 7 to 15 mm from the top of the sheet. Remove the TLC sheet and mark the solvent front immediately. After drying, view the TLC under the UV lamp and circle the spots. Calculate the R_f value of each spot. *Caution!* Ultraviolet light can damage the eye. *Never* look directly into the ultraviolet lamp.

Compare your results with those of the other students in the group. Determine which solvent give the best separation (different R_f s) of the standards (particularly those that appear to be in your analgesic sample). Each student should then use the third TLC plate with the best solvent to compare your sample with the standards that appear to be (or might be) in your sample. Spot the third plate as indicated below (you may spot your unknown once or twice).