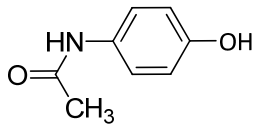
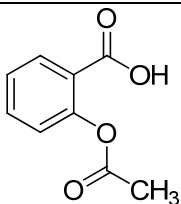
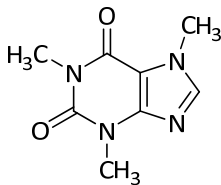
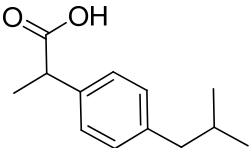
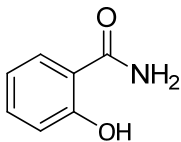


Thin-Layer Chromatography of Analgesics

Introduction

In this experiment, you will use thin-layer chromatography (TLC) to determine the composition of an unknown mixture of analgesics. You will be given five known reference substances, an unknown consisting of a mixture of three of the references, as well as one unknown containing a single reference compound. The reference samples are:

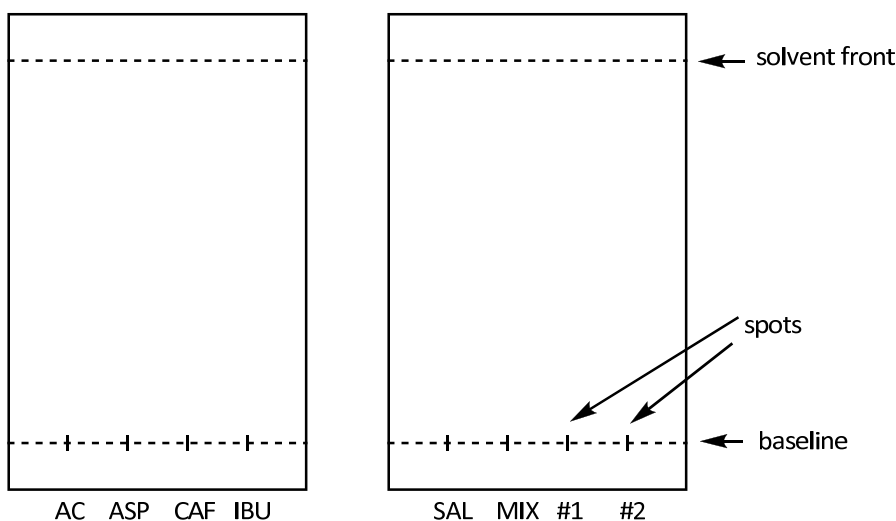
Acetaminophen (AC)	
Aspirin (ASP) (also called acetylsalicylic acid)	
Caffeine (CF)	
Ibuprofen (IBU)	
Salicylamide (SAL)	

The reference samples are all available as solutions consisting of 1 g sample dissolved in 20 mL of a 1:1 mixture of methylene chloride and ethanol. The unknown mixture and the single unknowns are similarly prepared.

Procedure – Spotting the TLC Plates

Working with a partner, obtain two TLC plates coated with silica gel and eight microcapillary tubes for spotting. **With pencil**, mark a baseline approximately 1 cm from the bottom of each TLC plate. Mark four lanes on each plate (see illustration below) and indicate what will be spotted on which lane. You will be spotting the five reference compounds, the unknown mixture, and two single unknowns (yours and your partner's). The lanes should be at least 0.5 cm from the side of the plate, and at least 1.25 cm apart.

Use the microcapillary tubes to spot the reference compounds, the mixture, and the two unknowns on the two plates. Be sure to use a clean microcapillary for each separate compound; please do not contaminate the reference compounds, the mixture, or the unknowns! If you are not sure if you have spotted enough compound on the plate, look at it under the UV lamp. You should see a dark purple spot on the baseline about 1-2 mm in diameter. If you can't see it, then you need to spot more compound there.



Procedure – Developing the TLC Plates

Obtain a 16 oz. wide-mouth screw-cap jar for use as a developing chamber. Fill the chamber with the developing solvent (0.5% acetic acid in ethyl acetate) to a depth of 0.5 cm (this may already have been done for you). Note that the solvent level must not be above the baseline, or the samples will dissolve

off of the TLC plate into the solvent pool rather than eluting up the plate as desired! Place both spotted TLC plates in the jar (see photograph below) and allow them to develop.

When the solvent has risen to a level about 0.5 cm from the top of the plate, remove the plates from the developing chamber and immediately draw a line in pencil on the plate at the solvent front. Put the plates on a paper towel on the bench top and allow the solvent to evaporate.

When the plate is dry, observe it under a UV lamp. Lightly outline with a pencil all of the spots that you see on the plate. Also note any particular colors or unusual fluorescence as this may assist in distinguishing compounds with similar R_f values. Trace the TLC plates and associated spots into your laboratory notebook, and note any distinguishing features.

Calculate the R_f values for each spot. Identify the components of the mixture, and identify each of the two single unknowns (be sure to record the unknown number in your notebook).



Figure 1 – TLC Jar with Two Plates

Questions

1. Why might it be difficult to distinguish *cis*- and *trans*-2-pentene by TLC?
2. What problem will arise if the level of the developing solvent in the chamber is higher than the level at which a spot is applied to the TLC plate?

3. What would you expect to see if a TLC plate has been left in the developing chamber too long (so that the solvent front has reached the top of the TLC plate)?
4. What will the result be of spotting too much compound onto the TLC plate?
5. One of the analgesics contains a chiral center. Which one is it? One of the two enantiomers is far more effective at reducing pain than the other. Which one?

Over-The-Counter Product	Major Components
Aspirin	Aspirin! (Acetylsalicylic acid)
Excedrin	Acetaminophen, Aspirin, Caffeine
Tylenol	Acetaminophen
Advil	Ibuprofen
Motrin	Ibuprofen
Anacin	Aspirin, Caffeine
Bufferin	Aspirin
Aleve	Naproxen