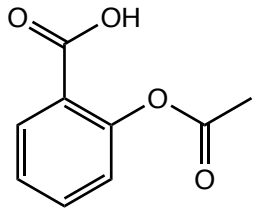
Isolation of the Active Ingredient of an Unknown Analgesic Drug

Objective

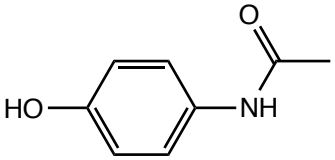
To extract the active ingredient of an unknown analgesic drug by correctly using several important techniques in organic chemistry and to determine the identity of the unknown analgesic by its melting point.

Background

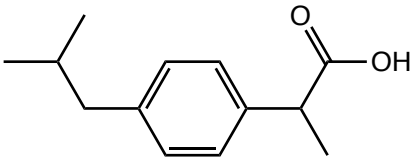
Most analgesic (pain-relieving) drugs found on the shelves of any drug or grocery store generally fall into one of four categories. These drugs may contain acetylsalicylic acid, acetaminophen, or ibuprofen as the active ingredient, or some combination of these compounds may be used in a single preparation. All tablets, regardless of type, contain a large amount of starch or other inert substance. This material acts as a binder to keep the tablet from falling apart and to make it large enough to handle. Some analgesic drugs also contain caffeine or buffering agents. In addition, many tablets are coated to make them easier to swallow and to prevent users from experiencing the unpleasant taste of the drugs.



Acetylsalicylic Acid



Acetminophen



Ibuprofen

Table 1. Analgesics and their melting points

Drug	MP	Brand Names
Acetylsalicylic Acid	135 – 136 °C	Aspirin, ASA, Emperin
Acetaminophen	169 – 170.5 °C	Tylenol, Datril, Panadol
Ibuprofen	75 – 77 °C	Advil, Brufen, Motrin, Nuprin

The purpose is to demonstrate some important techniques in organic chemistry and use them to determine the identity of an unknown analgesic. More specifically, you will extract (dissolve) the active ingredient of an unknown analgesic drug by mixing the powdered tablet with a solvent, methanol. Two steps are required to remove the fine particles of binder which remain suspended in the solvent. First, you will use centrifugation to remove

most of the binder. Second, you will use column chromatography as a purification technique to remove the rest of the binder from the active ingredient. Finally, the solvent will be evaporated to yield the solid active ingredient which will be collected on a Hirsch funnel. The identity of the unknown analgesic will be determined by the melting point of its active ingredient.

Procedure

If isolating: Analgesic 1, use one tablet in this procedure.
Analgesic 2, use one tablet in this procedure.
Analgesic 3, use two tablets in this procedure.

Extraction of the Active Ingredient

1. Using a pestle, crush the tablet(s) between two pieces of weighing paper. If the tablet is coated, try to remove fragments of the coating material with forceps after the tablet is first crushed.
2. Add all the powdered material to a 3-mL conical vial. Add 2 mL of methanol to the vial.
3. Cap the vial and thoroughly mix by shaking. Loosen the cap at least once during the process to release any pressure built up in the vial.
4. Allow the undissolved portion of the powder to settle in the vial. A cloudy suspension may remain even after 5 minutes or more. You should wait only until it is obvious that the larger particles have settled completely.
5. Using a Pasteur pipette, carefully transfer only the liquid phase to a centrifuge tube.
6. Add a second 2 mL portion of methanol to the conical vial, and repeat the shaking process as described above.
7. After the solid has settled, carefully transfer the liquid phase to the centrifuge tube containing the first extract.
8. Place the centrifuge tube in a centrifuge that is properly balanced and centrifuge the mixture for 3 minutes. The suspended solids should collect at the bottom of the tube leaving a **clear or nearly clear** supernatant fluid, the liquid above the solid. If the liquid is still cloudy, repeat the centrifugation.
9. Being careful not to disturb the solid at the bottom of the tube, transfer the supernatant liquid with a Pasteur pipette to a small test tube.

Column Chromatography

1. Prepare a silica gel column using a Pasteur pipette as follows:
   a. Insert a small ball of cotton into the top of the column and carefully push it down onto the neck of the pipette.
   b. Add about 2.0 cm of silica gel to the pipet and tap the column with your finger to pack the silica gel.
   c. Clamp the pipette in a vertical position so the liquid can drain from the column into a 5-mL conical vial.
2. Add 2 ml of methanol to the top of the column and allow it to drain until the level of methanol reaches the top of the silica. Do not allow the methanol to drain below the surface of the silica gel. If necessary, use more methanol.
3. When the level of methanol reaches the surface of the silica gel, transfer the solution containing the drug from the test tube to the column using another Pasteur pipette.
4. Collect the liquid that passes through the column into a 5-mL conical vial.

5. When all the liquid has passed through the column, add an additional 1 mL of methanol to the column and allow it to drain. This ensures that all the drug has been eluted from the column.

Evaporation of Solvent

Important: The evaporation procedure MUST be completed in 10-15 min to avoid decomposition of the drug.

1. Using a Pasteur pipette, transfer about half of the liquid in the 5-mL conical vial to a clean small test tube. Keep it safely for later use.
2. Evaporate the methanol in the 5-mL conical vial using a water bath at about 65oC. To speed the evaporation, direct a gentle stream of dry air into the vial containing the liquid.
3. Evaporate the solvent until the volume is less than 1 mL. Then, add the remainder of the liquid from the test tube and continue evaporation.
4. When evaporation is complete or it is apparent that the remaining liquid is no longer evaporating (volume less than 0.5 mL), remove the vial from the water bath and cool it to room temperature.
5. If liquid remains, place the vial in an ice bath carefully making sure the vial does not tip over. Crystallization may occur more readily if you scrape the inside of the vial with a spatula or glass rod.
6. If the solid is very hard or clumped together, use a microspatula to break up the solid as much as possible before the next step.

Vacuum Filtration

1. Set up a Hirsch funnel for vacuum filtration. Moisten the filter paper with a few drops of methanol and turn on the vacuum.
2. Use a microspatula to transfer the material in the conical vial to the Hirsch funnel.
3. Allow the crystals to dry for 5-10 min on the Hirsch funnel.
4. Carefully scrape the dried crystals from the filter paper into a tared (previously weighed) watch glass. If necessary use a microspatula to break up any remaining large pieces of solid .
5. Allow the crystals to air-dry on the watch glass.

Analysis

1. Weigh the watch glass to determine the mass of your unknown analgesic.
2. Use a small sample of the crystals to determine the melting point range of your unknown analgesic. You may observe some “sweating” or shrinkage before the substance actually begins to melt. The beginning of the melting point range is when actual melting is observed, not when the solid takes on a slightly wet or shiny appearance or when shrinkage occurs. Record the temperature when the first drop of liquid is observed and the temperature at which the final bit of solid melts.
3. Determine the identity of your unknown analgesic by comparing its melting point to the options on Table 1.
4. **Save your sample for thin layer chromatographic (TLC) analysis next period**. You may also wish to perform a second melting point analysis next week.

Thin Layer Chromatographic Analysis of an Unknown Analgesic

Objective

To analyze and identify an unknown analgesic using thin layer chromatography.

Background

Thin layer chromatography (TLC) is based on the partitioning of an analyte between a mobile and stationary phase. The phenomenon behind TLC can be seen when some inks get wet and are separated into their component colors as the water diffuses into the paper the inks were applied to. Inks that dissolve readily water tend to move with the water as it diffuses into the paper. The inks that are strongly attracted to the surface and do not dissolve in the water do not move with the water. The difference between the solubility of the component inks and their attraction to the paper, thus, causes the inks to separate. In this example, the paper is the stationary phase, the water is the mobile phase, and the ink is the analyte.

TLC is typically done with a thin layer of alumina or silica gel applied to a plastic or aluminum support. Organic solvents are typically used instead of water. Regardless, the idea remains the same: organic materials that are more strongly attracted to the stationary phase move more slowly as the solvent diffuses across the plate than the materials that are less strongly attracted to the stationary phase. Of course, the choice of solvent is important too. The solvent must be able to dissolve the components in the analyte or they will not move across the plate. On the other hand, if all of the components are extremely soluble in the solvent, they may all move together, and no separation would occur.

In this activity, a suitable solvent mixture and stationary phase have already been chosen.

Procedure

Overview: your unknown will be applied to a TLC plate and three known analgesics will also be applied to the plate. The TLC plate will be placed in a developing chamber and the solvent at the bottom of the developing chamber will be allowed to diffuse up the TLC plate until it almost reaches the top of the plate. Once the TLC plate has dried, the locations of your unknown and the known compounds can be visualized using UV-light. By comparison to the known compounds, the unknown can be identified based on the distance that it travels. Of course, one chromatogram is not sufficient to unambiguously determine the identity of a compound, but it can be used with other evidence, like a melting point, to support the identification of a compound.

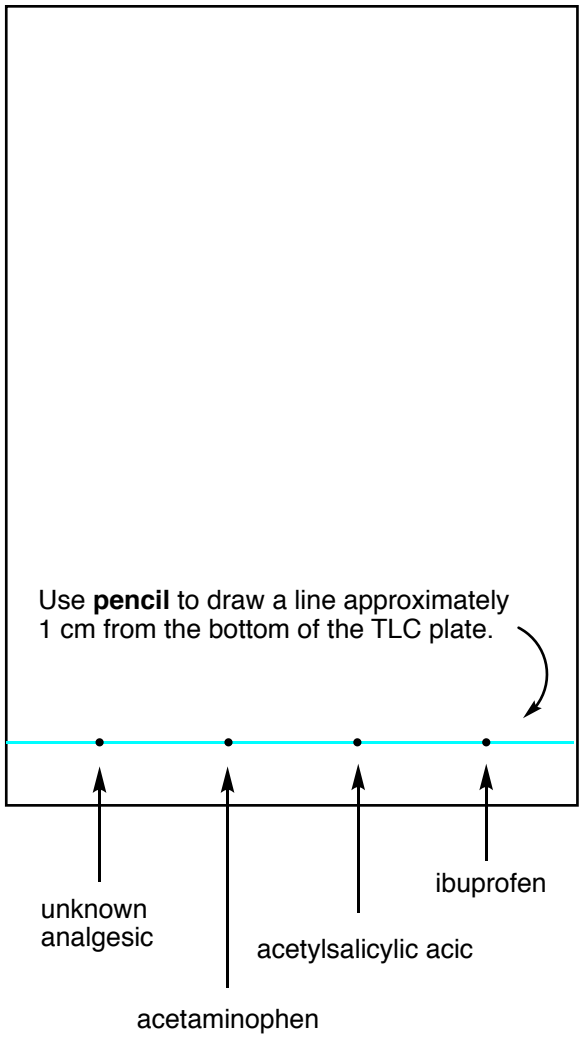
Preparing a TLC Plate
Obtain a TLC plate and capillary micropipets (made from open ended melting point capillary tubes). It is important to handle the TLC plate gently and by the edges.

Using pencil and a straight edge, gently draw a line across the TLC plate approximately 1 cm from the bottom edge of the plate. Place 4 marks across the line to indicate where you will spot the samples.

Creating a Developing Chamber
Collect a 600-mL beaker, a piece of filter paper, and a watch glass. Fold the filter paper into an L-shape, and place it in the 600-mL. Add enough of the ethyl acetate/acetic acid developing solution to cover the bottom of the beaker to a depth of about 5 mm. The level of the solvent must stay below the the spots that are placed on the TLC plate. Wet the filter paper with the developing solution and place a watch glass over the beaker. Allow the developing solution to evaporate into the air inside the covered beaker while spotting the TLC plate.

Spotting the Analytes
Dissolve approximately 10 mg of your unknown analgesic in a 1 mL of methanol. Draw the solution into the micropipet by touching the pipet to the solution; capillary action will draw the liquid into the pipet. Gently and briefly touch the micropipet to the previously marked position on the TLC plate. The solution will be drawn onto the plate. Make certain to remove the micropipet before the spot becomes wider than 2 mm. Allow the solvent to evaporate and repeat two times. The spots tend to spread as they move, so smaller spots give better results.

Spot the known materials on the previously marked positions on the plate following the procedure as described above. Make certain to note the order that you place the spots on the plate.



Sample TLC Plate

Develop the Plate
Place the TLC plate into the beaker making certain that the edges of the TLC plate do not touch the filter paper liner otherwise solvent will flow into the plate from the area where the plate and the liner are touching. Place the watch glass back on top of the beaker and allow the solvent to diffuse up the TLC plate. When the solvent comes to within 0.5 cm of the top of the plate remove the plate from the developing chamber and with a pencil gently draw a line across the solvent front.

Place the TLC plate in the hood and allow the solvent to evaporate from the plate.

Visualize the Results
In a darkened hood, shine the light from a UV lamp onto the TLC plate. The plate will fluoresce. The compounds will appear as dark spots. (Some compounds may also fluoresce, but they would still stand out from the fluorescence of the TLC plate.) Circle the positions on the materials on the plate.

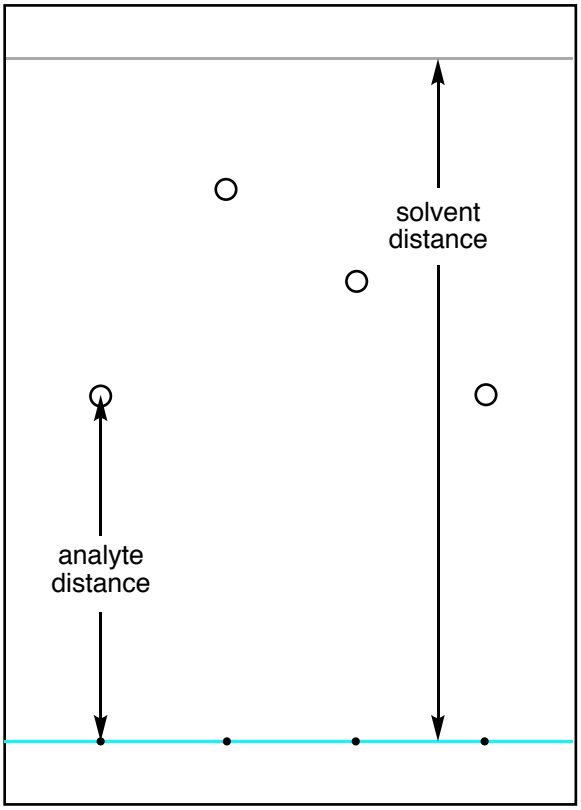
Determine the R_f Values
The R_f value (retardation factor) of a compound is the distance that the spot traveled (measured to its center) divided by the distance that the solvent traveled.

Remember to record the masses of the active ingredients in each of the analgesics so you can use that information while preparing your report.

Report
In a typed report, tabulate the R_f values for your unknown analgesic and the three known analgesics and report the melting point of your analgesic. Based on these data determine the identity of your unknown analgesic and using that information look up the mass of the active ingredient in the analgesic pill.

Report the mass of analgesic that you isolated, the number of moles or millimoles of analgesic isolated, the percent recovery that you achieved based on the mass of active ingredient found above. Remember to also report the appearance of the active ingredient.[1]

Open ended response: In a few sentences suggest possible advantages to using TLC in addition to melting points to determine the identity of the unknown analgesic.



$$R_f = \frac{\text{analyte distance}}{\text{solvent distance}}$$

Determining the R_f values on a developed TLC plate.

[1] If time did not permit you to isolate the active ingredient or if something went horribly wrong, consult with your instructor. You may be asked to attempt a second isolation or report results based on data provided by the instructor.