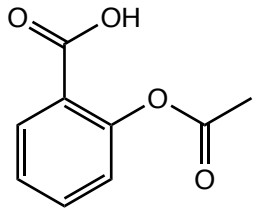
Isolation of the Active Ingredient of an Unknown Analgesic Drug

Objective

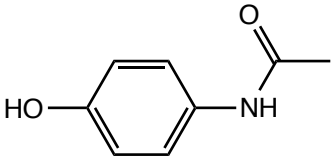
To extract the active ingredient of an unknown analgesic drug by correctly using several important techniques in organic chemistry and to determine the identity of the unknown analgesic by its melting point.

Background

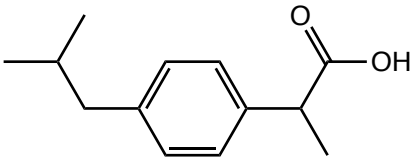
Most analgesic (pain-relieving) drugs found on the shelves of any drug or grocery store generally fall into one of four categories. These drugs may contain acetylsalicylic acid, acetaminophen, or ibuprofen as the active ingredient, or some combination of these compounds may be used in a single preparation. 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**.

Report

The report is described at the end of the TLC activity, and it is due after completing the TLC activity.