Separation of the Components of an Analgesic Tablet I: Acid-Base Extraction

Introduction

Acid-base extraction will be used to separate the components of extra-strength Excedrin®, or a generic brand of the same composition, a combination pain reliever that contains three active ingredients: acetylsalicylic acid (aspirin), acetaminophen, and caffeine. The components will be isolated, analyzed for purity by thin-layer chromatography and their percent recoveries determined.\(^1\)

Background

One tablet of extra-strength Excedrin contains aspirin (250 mg), acetaminophen (250 mg), and caffeine (65 mg) as well as a binding material. The combination of these three compounds is said to be effective against migraine headaches.\(^2\)

Aspirin, an analgesic and anti-inflammatory, is the most used drug in the world, with an annual global consumption of 40,000 tons.\(^3\) Caffeine, a stimulant, is the most consumed psychoactive substance in the world and one that 90% of North Americans consume on a daily basis.\(^3\) Acetaminophen is an analgesic and antipyretic that is sold as the brand name Tylenol\(^\text{®}\) as well as being present in several combination products in addition to Excedrin.\(^2\)

Acid-base extraction separates the three compounds because they have different acidities. Aspirin is the most acidic, with \(pK_a\) 3.5, followed by acetaminophen with \(pK_a\) 9.9, and then by caffeine which is effectively neutral. After the tablet is dissolved in ether and the insoluble binder is removed, the ether solution is extracted first with aqueous \(\text{K}_2\text{HPO}_4\) to separate out the conjugate base of the aspirin and then with aqueous \(\text{KOH}\) to separate out the conjugate base of the acetaminophen. The caffeine remains unchanged in the ether layer and is isolated from that layer by evaporation of the ether (after first removing traces of water by using \(\text{Na}_2\text{SO}_4\)). The aspirin and acetaminophen are precipitated from their respective solutions by acidifying each with \(\text{HCl}\) and the precipitates are isolated by vacuum filtration. Each isolated solid is weighed and analyzed for purity by thin-layer chromatography. The percent recovery of each component will be calculated based on the amount of that component that is alleged to be present in the tablet.

According to the original source\(^1\) aspirin recoveries are typically 60% or higher. Acetaminophen recoveries are <10% due, in part, to the fact that it often forms a fine solid that is difficult to filter. Caffeine is typically recovered in <5% due to the lower solubility of caffeine in diethyl ether (1.8 mg/mL) than in water (22 mg/mL). The solubility of caffeine in water also means that both the acetaminophen and the aspirin isolated via extraction may be contaminated with caffeine.

If you also do the chromatographic separation of the components of a tablet, your instructor may have you compare the two separation techniques with respect to the percent recoveries and purities of the three substances isolated.

Additional Reading


Techniques (from Techniques in Organic Chemistry by Mohrig, et al., 4th edition)

- Essay on Learning to Do Organic Chemistry, page 41
  - Measurements: Chapter 5.
- Essay on Intermolecular Forces, page 127
  - Vacuum Filtration: Section 9.4.
  - Extraction: Chapter 10, except section 10.6.
  - Drying and Recovering Products: Chapter 11 (no rotovap).
- Essay on Chromatography, page 253
  - Thin-Layer Chromatography: Chapter 18.
**Equipment**
- Mortar and pestle, or pill grinder
- Separatory funnel with glass stopper; iron ring
- pH paper
- TLC plates; uV lamps; TLC spotters (micropipetters; in cylindrical cardboard container); developing chamber

**Safety Information**
- Acetaminophen, N-(4-hydroxyphenyl)acetamide, is slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.
- Acetic acid is very hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, of inhalation. Hazardous in case of skin contact (corrosive, permeator), of eye contact (corrosive). Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract.
- Acetone is hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator). Flammable with flash points of -20°C (closed cup) and -9 (open cup).
- Acetylsalicylic acid (aspirin) is hazardous in case of skin contact (irritant), of eye contact (irritant). Slightly hazardous in case of skin contact (corrosive, permeator), of ingestion, of inhalation. Severe over-exposure can result in death.
- Caffeine is toxic and an irritant in its solid form. Avoid contact with skin, eyes, and clothing. Mutagenic for mammalian somatic cells. May be toxic to heart, gastrointestinal tract, central nervous system (CNS). Repeated or prolonged exposure to the substance can produce target organs damage.
- Diethyl ether, also known as ether, is very flammable and volatile with a low flash point (-45°C, closed cup). Use in a hood if possible. Keep away from hot electrical devices. Do not use anywhere near an open flame. Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator).
- Ethyl acetate is hazardous in case of ingestion or of inhalation. Slightly hazardous in case of skin contact (irritant, permeator), of eye contact (irritant). Flammable with flash points of -4.4°C (closed cup) and 7.2 (open cup).
- Hexanes are hazardous in case of skin contact (permeator), of ingestion, of inhalation. Slightly hazardous in case of skin contact (irritant), of eye contact (irritant). May be toxic to peripheral nervous system, skin, central nervous system (CNS). Flammable with a flash point of -22.5°C (closed cup).

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**Student Safety Summary - Diethyl Ether**

- **Precautions**: Keep away from hot surfaces and flames. Use a water bath with hot tap water and no other heat source when evaporating actively from a container. Use in a fume hood. Cap or stopper any container that is used to store or transport even small quantities.
- **Standard PPE** plus nitrile or neoprene gloves and flame-resistant lab coat.
- **First Aid** Move into fresh air if inhaled. Wash off skin with soap and water for at least 15 minutes. Rinse eyes with plenty of water for at least 15 minutes.
- **Waste** Place unused ether or mixtures containing ether in the container for non-halogenated organic waste.

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- Hydrochloric acid solution is a skin irritant. Avoid contact with skin, eyes, and clothing.
- Potassium hydrogen phosphate, K2HPO4, is hazardous in case of eye contact (irritant), of ingestion. Slightly hazardous in case of skin contact (irritant), of inhalation.
- Potassium hydroxide is very hazardous in case of skin contact (corrosive, irritant), of eye contact (irritant, corrosive), of ingestion, of inhalation. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness.
- Silica (silica gel, SiO2•xH2O) is slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of
ingestion, of inhalation.

- Anhydrous sodium sulfate (drying agent) is hazardous in case of eye contact (irritant). Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation.

**Procedure**

**A. Separation by Extraction (see flowchart on the next page)**

1. Crush three Excedrin® or Excedrin-like tablets (note brand!). Transfer the powder to a beaker and then add 25 mL of diethyl ether and stir. The three active components will dissolve, but the starch binder will not. Carefully decant the ether mixture away from the solid binder into a small flask or the separatory funnel. If you are concerned about decanting, do a gravity filtration instead. Rinse the solid with 10 ml of diethyl ether and add the ether rinse to the ethereal solution.

2. Extraction
   a. Obtain 10 mL of 1M K_2HPO_4 and record its pH. Transfer the ether solution to the separatory funnel (if not already there) and add the 10 mL of 1M aq. K_2HPO_4. Mix the layers as described in section 10.3 and illustrated in Figure 10.4 of the Techniques book. Make sure you know which layer is which. The aqueous layer should be drained into a small labeled container and set aside.
   b. The ether layer should still be in the separatory funnel. To this layer, add 10 mL of 1M KOH after first recording the pH of the KOH solution. Mix the layers again. If you get an emulsion let the mixture sit in the separatory funnel for a few minutes. This aqueous layer should be drained into a second small labeled container and set aside.

3. Drying (removal of water)
   a. Transfer the ether layer to a small labeled Erlenmeyer flask, add enough anhydrous sodium sulfate to remove traces of water (see Figure 11.1 in Techniques book, along with the accompanying text).
   b. Transfer the ether solution (typically by decanting, or you may do a gravity filtration) making sure to leave the solid sodium sulfate behind) to a pre-weighed beaker or Erlenmeyer.

4. Isolation of compounds
   a. Caffeine. Evaporate the ether in a warm-water bath (use hot tap water, NOT the hot plate!) or by setting it in the hood while you do the remaining steps. This should yield the pure neutral compound (caffeine) as a solid material. Do NOT use a flame or even a hot plate to evaporate the ether! Yields of caffeine are typically low and it may appear as a film in the beaker. Determine the mass of the caffeine (if you can--there may be too little to weigh).
   b. Aspirin. Determine the pH of the biphosphate extract and then add 6M HCl dropwise until the solution is acidic (use pH paper as follows: dip a clean stirring rod into the solution and then touch it to a piece of pH paper on a paper towel; you want a red color, or pH 1-2, in this case). Solid aspirin should crystallize out of solution; keep adding acid until no more solid forms when you add more HCl. Note the approximate number of drops of HCl needed and the final pH. Chill the solution and crystals in an ice bath. Collect the product by vacuum filtration using a Hirsch funnel (Technique 9.4, Figure 9.8a - left), and allow it to air dry, preferably until the next lab period.
   c. Acetaminophen Determine the pH of the hydroxide extract and then acidify it to pH 1-2 with 6M HCl to precipitate the acetaminophen. Note the new pH and the approximate number of drops of HCl. This compound doesn’t always come out of solution and is often not produced in large quantities. Try chilling it in the ice bath. Collect crystals by vacuum filtration (unless there seems to be a small amount—consult with instructor) and allow to air dry until the next lab period.

5. Measure the mass of each component (next lab period, after allowing them to dry). Save samples for TLC analysis (see B.8).

6. **Cleanup**: all organic waste should go in the organic waste container. All aqueous waste should go in the acid/base waste container. Put used pH paper, filter paper, and drying agent in the solid waste container.
B. Thin-Layer Chromatography
You may do the Reference TLC at any point; however, you need the separated components to do the TLC of the Isolated Components and this may not be until the second lab period. The Reference TLC is the same as part A of the Analgesic II Lab (separation by chromatography); your instructor may have you do it only once, even if you are doing both experiments.

1. Reference Compounds and Tablet
   a. You will be provided with reference solutions of each of the three pure compounds and of the tablet, each dissolved in acetone.
   b. Obtain a 2 x 5 cm TLC plate. Lightly draw a pencil line 1 cm from the bottom of the plate. Lightly draw four hatch marks, evenly spaced across the starting line. Label the hatch marks 1-4. (Technique 18.2). Don’t draw a line near the top.
   c. Use a micropipet to deposit a tiny amount of solution of each reference compound solution on one of the first three marks. On the last hatch mark, spot a tiny amount of the solution of the soluble components of the tablet. Keep track of which is which! Look at the

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*Flowchart for Analgesic Extraction*

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undeveloped plate under the uV lamp to make sure that there is enough of each material to see, but that the spots are not too large. If the spots are not visible, then spot over them a few times each, allow the spot to dry each time before spotting again.

d. Use a 100-mL beaker to prepare a developing chamber containing <1 cm depth of a solution of 1:2 hexanes:ethyl acetate with 1% acetic acid. Make sure you have a lid to cover the jar (a watch glass, aluminum foil, or stretchy plastic wrap work well). (Technique 18.3)

e. Place the TLC plate in the developing chamber using forceps. Allow it to remain undisturbed in the covered chamber until the solvent is nearly to the top of the plate (3-5 mm from the top).

f. Remove the TLC plate. Immediately lightly draw a pencil mark to indicate the location of the solvent front, then wait for about 20 seconds to allow the solvent to evaporate.

g. Use the UV light to visualize your spots (Figure 18.6). Use a pencil to trace the location and size of your spots. Record a sketch of your TLC plate in your notebook. Measure the distances traveled by each spot and by the solvent front and include those in the sketch. Calculate the $R_f$ of each spot (Technique 18.5).

h. Expected approximate $R_f$ values are as follows: aspirin-0.6, acetaminophen-0.3, and caffeine-0.1

2. Isolated Components

   a. In three separate test tubes, dissolve a small amount of each compound isolated from the extraction (Step A.4) in a few drops of reagent-grade acetone. If you only have a tiny amount of any solid add a few drops of acetone directly to the beaker that contains the solid and swirl it around to dissolve the solid.

   b. Use TLC to analyze each component using the above procedure starting with step 1.b, again using four hatch marks, one for each isolated compound and the fourth for the reference mixture of the three compounds. Don’t forget to check that you have enough material in each spot with the uV lamp before you develop the plate.

   c. Develop and visualize the plate. Record a sketch of your TLC plate in your notebook. Measure the distances traveled by each spot and by the solvent front and include those in the sketch. Calculate the $R_f$ of each spot (Technique 18.5).

3. Cleanup: all solvents and TLC samples go in the organic waste. Used TLC plates and pH paper go in the solid waste container (if there is one, it is usually a large beaker) in the hood next to the other waste containers. Micropipetters go in the same place as the used TLC plates or in the broken glass container.

Practice Questions

1. Suppose a student extracted the ether solution containing all three compounds first with the KOH solution and then with the $K_2HPO_4$ solution. Draw structures of the substance(s) present in each of the following: the KOH extract, the $K_2HPO_4$ extract, and the ether solution.

2. Give one reason why the recovery of caffeine is expected to be low.

References

(1) Revell, K. D. J. Chem. Educ. 2011, 88, 1413-1415. This is the source of this laboratory experiment.