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Forensic analysis of analgesics by TLC

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Forensic Analysis: TLC of an Analgesic

Introduction:

Analgesics are a class of chemical substances that are found in many over-the-counter drugs that alleviate pain. However, despite their similar therapeutic effects, the active ingredient in formulations such as Excedrin, Advil, and Tylenol is highly variable. Aspirin, for example, has a chemical structure unrelated to both ibuprofen and acetaminophen and yet it relieves pain just the same. Since the structures of analgesics are different, it is likely that they also have different chemical properties. Judging by the presence of diverse functional groups in these molecules, one way they will differ is their overall polarity.

In order to study polarity differences, however, we must first separate the analgesics from the other components in the tablet mixture to obtain pure compound. Most analgesics come in easy-to-handle tablet form which is a combination of the active ingredient plus inert substances such as starch, sucrose, caffeine, salt, and/or binding agents. Many tablets also contain an external coating that makes them easy to swallow and which readily breaks apart in the body, releasing the active drug. In this laboratory experiment, you will isolate the active ingredient of an unknown analgesic and then identify the unknown by analyzing its polarity using a technique called thin layer chromatography.

Consider the following scenario for this experiment:

You are a forensic chemist who has been testing evidence from the scene of a crime. A young male was found beaten and unconscious in a dark alley. The victim remains in a coma and preliminary toxicology tests indicated that no foreign substances were present. However, police detectives recovered an unlabeled pill near the victim and suspect that he may have been the victim of a drug deal gone wrong. You need to help the detectives by determining the identity of the active ingredient in the pills, so that potential leads may be followed to apprehend the suspect.

References:

Forensic Analysis: Thin Layer Chromatography of an Analgesic

Equipment:
- Microscale glassware kit
- Pasteur pipets
- Capillary tubes
- TLC plates
- Cotton

Chemicals:
- One tablet of an unknown analgesic, labeled I, II, or III.
- Solution of aspirin in methanol (1 g in 20 mL), labeled aspirin
- Solution of acetaminophen in methanol (1 g in 20 mL), labeled acetaminophen
- Solution of ibuprofen in methanol (1 g in 20 mL), labeled ibuprofen
- Methanol
- Methylene chloride
- 0.2% acetic acid/ethyl acetate mixture (100 mL)
- Ethyl acetate
- Alumina


Extraction:
1. Obtain an unknown sample from your instructor. Crush your tablet(s) between two pieces of weighing paper using a mortar or spatula until it is a fine powder. Transfer to a 3 mL conical vial and add about 2 mL of methanol.
2. Cap the vial and shake thoroughly to mix several times. Loosen the cap to release any pressure the builds up in the vial. Allow the solid particles to settle to the bottom (a cloudy mixture will result). With a Pasteur pipet, decant the liquid into a Falcon tube, being careful not to disrupt the solid that has collected at the bottom.
3. Repeat step 2 with an additional 2 mL methanol. Label your tube with your initials and then find a partner. Centrifuge both samples (must be roughly equal in mass: use the balances!) for 2-3 minutes. Transfer the supernatant to a test tube.
Isolation:
1. Insert a small ball of cotton into the wide end of a Pasteur pipet and use a wooden stick to pack it down into the narrow spout. Add about 0.5 g of alumina to the pipet and tap the column with your fingers to pack the column tightly.
2. Clamp the pipet vertically and place a small tared beaker underneath. Add about 2 mL methanol via pipet into the top of the pipet column and allow it to drip into the beaker using a pipet bulb to add pressure to the top of the column. Discard this methanol.
3. When the solvent level has reached the top of the alumina, remove the pipet bulb without releasing your finger pressure to ensure that air is not drawn up through the bottom of the pipet. Then, transfer your solution containing your unknown analgesic to the top of the column and collect it in the same tared beaker as before. Do not let the alumina run dry. Finally, rinse the column with another 1 mL of methanol and collect it in the same beaker.
4. In your fume hood, take the beaker containing your pure analgesic and remove the methanol by evaporating it in a water bath set to 50 °C using a gentle stream of air. Cool it to room temperature once all the methanol has been evaporated and obtain the weight of your beaker.

NOTE: Methanol is flammable. Extra precaution should be taken to avoid contact with sources of ignition. Always monitor your evaporation.

Identification of the unknown:
1. To prepare a solution of your sample, dissolve your unknown in 10 mL 1:1 methanol:methylene chloride. Make sure to label this beaker.
2. Obtain a few silica gel TLC plates from your instructor. Be careful not to bend the plate because the flexible backing may cause some of the silica gel to flake off.
3. In pencil, lightly draw a line across the plate about 0.7 – 1.0 cm from the bottom of the plate, parallel to the narrowest edge of the plate. This will be your baseline. Perpendicular to this line, make three tick marks spread evenly across this line. These marks will eventually become spotted with different samples, so label them accordingly with a letter or number.
4. In a capillary tube, draw up a solution containing your unknown and spot it lightly on the plate at the left tick mark until the solvent comes out (it may not be necessary to spot the entire amount in the capillary tube). Allow the solvent to evaporate. Repeat again in the middle tick mark – this will be called the “cospot.” Repeat for one of the three known analgesic standards, this time in the right and middle tick marks.
5. Since all of these samples can be detected by UV light, check to make sure you have enough analyte on the plate by looking at the plate under UV light. You should see a black/blue spot if you have enough compound on the plate. If not, add more. Likewise, if you have too much analyte on the plate, make a new plate with less compound (a more dilute solution).
6. Add about 5 – 10 mL of eluent (0.2% acetic acid/ethyl acetate) to the TLC chamber. Place a thin piece of filter paper inside the chamber to ensure vapor homogeneity.
7. Insert the TLC plate into the chamber, allowing it to rest evenly in the solvent. Be sure that the solvent level is below the baseline of your plate.

8. Develop the plate until the solvent is about halfway from the top of the plate and immediately draw a line in pencil. This line will indicate the solvent front.

9. Analyze your plate by irradiating the plate with UV light. Draw a circle around each of the spots you observe. If your plate does not look correct, rerun the TLC analysis.

10. Calculate the retention factor (R_f) of each of the samples by dividing the distance traveled up the plate by the distance traveled by the solvent front. Measure the R_f of each spot by using the center of the spot for your calculation. Use the pure sample for this calculation, not the cospot. (R_f = d_{analyte}/d_{solvent front})

11. Repeat until you have all three standards measured on three different TLC plates (each plate should also contain your unknown/cospot).

12. If you have time, repeat with 100% ethyl acetate as your elution solvent. Do you notice any difference in the R_f values? Why might this be the case?

Analysis: Obtain a percent recovery of the unknown analgesic and record the R_f values for each reference solution. Determine the identity of the unknown using this data.

Pre-Lab Questions:
1. Based on what you know about polarity, make a hypothesis about which analgesic will be the most polar and which will be the least polar.
2. Why does the tablet need to be crushed in the experiment? What is the purpose of the alumina column?
3. If you measure the relative mobility of a compound to be 0.75 in 20% methanol:80% methylene chloride, how would you decrease the R_f to be between 0.3-0.5?