Experiment 1 – Separation of Excedrin Components *via* Column Chromatography

Revell, K. D. *Journal of Chemical Education.* **2011**, 88, 1413.

Learning Objectives

- Understand principles behind silica-based chromatography
- Critical analysis of column and thin-layer chromatography technique
- Analyze data to assess purity and success of experiment
- Understand the role of functional groups and polarity on separation
- Predict sources of error and understand their effects on results

---

How to Prepare for Lab + Assignments - Follow Canvas Exp 1 Module…

Before Lab

- Read this PDF – background, procedure, safety, pre-lab and in-lab questions
  - Option: listen to Caitlin read this document in the **8M Exp 1 Podcast**
- Attend and/or watch **lab lecture** – we go over everything you need for your assignments!
- Practice the lab online *via* **Slugs@home platform** - sites.google.com/ucsc.edu/slugshome/home
- Complete the **pre-lab questions** at the end of this doc - incorporated into Canvas quiz ☺
  - **Quiz** due before your enrolled section – check Canvas for due date
- Download the **Exp 1 worksheet** on Canvas and prepare your **lab notebook**…

Lab Notebook Preparation – *worksheet = template / outline to copy by hand into lab notebook*

- **Purpose:** one-sentence summary of the main lab goals plus the structures of Excedrin components
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical properties!
- **Procedure with Diagrams** – complete before starting lab; sample on Canvas
  - Use the procedure that follows to create your hand-drawn experimental instructions
    - Simple sketches & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!
    - Avoid copying the procedure word-for-word.
    - Make it easy for anyone to follow your procedure without referring to this document.
  - **Slugs@home Exp 1 website** - Equipment & Safety pages; pictures & videos of the whole lab
  - The **class notes** include useful diagrams as well

During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A
- Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
  - Guidelines at end of this document

Background: Principles & Theory of Silica Chromatography

Column chromatography is a type of *adsorption chromatography* used to separate components from a mixture based on selective affinity to stationary and mobile phases. The principles of column chromatography and thin-layer chromatography (TLC) are analogous. The separation of components from a mixture is based on **polarity**. The *stationary phase* is most commonly silica (SiO_2) and the *mobile phase* is an organic solvent or solvent mixture. SiO_2 is polar and has a greater affinity for polar compounds. Thus, *less polar compounds will always elute from a column earlier and move farther on a TLC plate than polar compounds.*

In TLC, the mobile phase moves up the SiO_2 plate by capillary action, against gravity (**Figure 1a**). Less polar compounds travel farther up the TLC plate (higher retention factor, R_f). Components remain on the plate and are analyzed by visual inspection. In column chromatography, the mobile phase is added to the top of the column and travels down with gravity, eluting components as a solution in the solvent (**Figure 1b**). Less polar compounds elute from the column first due to their low affinity for SiO_2. Components can be isolated from the mixture in column chromatography, whereas TLC separates components without collection.

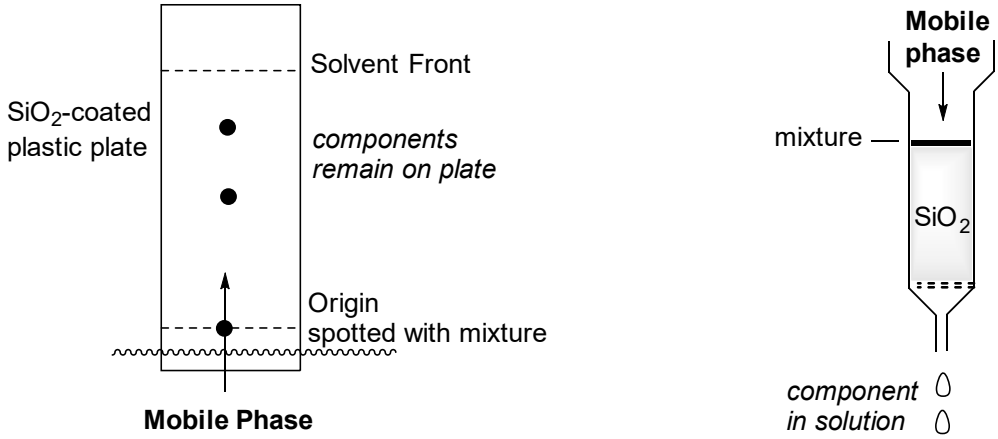

Figure 1. Diagrams of **(a)** TLC, **(b)** column chromatography

Students will use column chromatography to separate the active ingredients of Excedrin, an over-the-counter analgesic (**Figure 2**). A column is packed with SiO_2 then dry-loaded with an Excedrin-SiO_2 mixture. Increasingly polar solvents will be added to selectively elute aspirin (ASP), acetaminophen (ACE), then caffeine (CAF) based on their increasing polarities. This **solvent gradient** includes mixtures of hexanes and ethyl acetate (EtOAc) then the more polar acetone. Solutions of ASP, ACE, and CAF will be collected in fractions and analyzed by TLC. Pure fractions will be concentrated to isolate each component and compared to known values. Students will separate Excedrin components by a different method (acid-base extraction) and the results will be compared in Experiment 2.

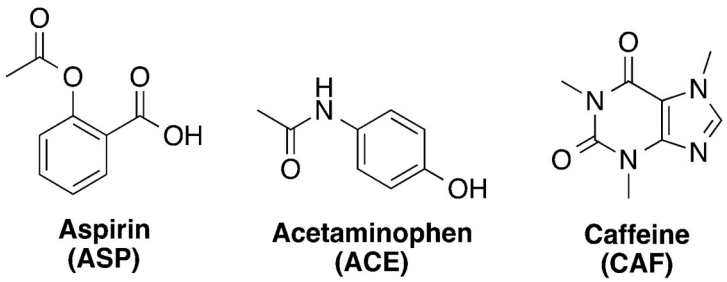

Figure 2. Active ingredients in Excedrin

PROCEDURE

1. Active Ingredients in Excedrin - Obtain the mass of an Excedrin® tablet then crush to a fine powder using a mortar and pestle. Add 20 mL of ethyl acetate (EtOAc) to the mortar and gently mix with a stir rod in the fume hood for 2 minutes. The three active components should dissolve but the starchy tablet binder (inactive ingredients) will not. Decant the solution into a small glass funnel with a small piece of cotton using a glass stir rod to aid in the transfer. Rinse with an additional 2 mL EtOAc. Collect the filtrate in a labeled 50-mL round-bottom flask (RBF).

2. Prepare & Load the Column - Add approximately 0.5 mL of SiO_2 to the RBF (compare to reference in the lab) and concentrate to dryness using a rotary evaporator (rota-vap). The result is Excedrin-coated SiO_2! Prepare the following solvent mixtures in the fume hood and keep in labeled, covered Erlenmeyer flasks. ***Prevent chemical exposure incidents and spills*** by using a funnel to transfer the majority of each solvent or mixture from the reagent container into a graduated cylinder, then use a pipet to bring the solution up to the proper volume.

(1) 30 mL of 1:1 hex / EtOAc = 15 mL hexanes, 15 mL ethyl acetate

(2) 30 mL of 1:2 hex / EtOAc = 10 mL hexanes, 20 mL ethyl acetate

(3) 35 mL of acetone

Label seven large test tubes for collection of the column fractions and keep in a test tube rack. Clamp a disposable polypropylene column (1.5 x 12 cm) to a ring stand and make sure there is a filter toward the bottom (add one if not present). Add approximately 3 g of SiO_2 to the column (reference provided in lab) followed by the Excedrin-SiO_2 mixture using a powder funnel. Add a level, ~2 mm layer of sand on top of the sample to prevent disturbing the stationary phase.

3. Run the microcolumn: Clamp the column to a ring stand in the fume hood and twist off the tip of the plastic column, if necessary. Carefully add the first solvent portion (1:1 hex / EtOAc) without disturbing the sand by *slowly swirling the pipet around the inside walls of the column* while gently applying pressure to the pipet bulb. Once the solvent level is high enough, the remaining solvent can be added more quickly. *Do not allow the column to run dry or test tubes to overflow*. Collect the eluent in the test tubes labeled “F1” and “F2,” switching approximately 15 mL per test tube. Switch to the next solvent mixture (1:2 hex / EtOAc) and collect each successive ~15 mL portion of solvent in test tubes “F3” and “F4.” Repeat with acetone, collecting in “F5” and “F6.” TLC analysis can be completed as fractions are obtained, space permitting.

4. TLC Analysis: *TLC analysis of the standards can be done at any time and shared as a lab*. Place a small amount (microspatula tip) of ACE, ASP, or CAF standard in a small test tube and dilute with 1 mL acetone. Analyze each column fraction by TLC in comparison to the standards. Carefully but quickly spot the plate once per each column fraction. These compounds are highly UV active and require only minimal amounts for visualization. The spot should be small enough for 2-3 lanes per plate without smearing. Rinse the capillary

tube with acetone to prevent cross-contamination between samples. Before placing the plate in the developing chamber, visualize the spots with a UV lamp to ensure you added enough sample to visualize but not so much to smear when run.

Keep the TLC chambers in the fume hoods and covered at all times. Use tweezers to carefully place the TLC plate into the developing chamber without disturbing the mobile phase. Place the cap on the jar upside down (screwing the cap on will likely disturb the mobile phase!). Allow the TLC plate to run until the solvent is approximately 1 cm from the top of the plate. Remove the plate with tweezers, quickly draw the solvent front on the plate, and wait until the solvent evaporates in the hood before visualizing with the UV lamp. Circle the spots then calculate all R_f values at your bench (distance of spot from origin / distance of solvent front from origin). Dispose of the plates in solid waste.

5. Isolation of Components: Any fractions containing a single compound as determined by TLC can be concentrated using a rota-vap (*in vacuo*). If two fractions contain the same single compound by TLC, those fractions can be combined. Transfer the fraction(s) to an appropriately sized, pre-weighed RBF and remove the solvent. The compound may or may not solidify on the rota-vap but do keep it on the vacuum for a few extra minutes to ensure complete removal of solvent. Record the mass of each component isolated and determine the percent recovery compared to the initial mass of Excedrin tablet. Experimental success will be determined by comparison to your responses in pre-lab #1 (theoretical percent recovery).

Clean-up	**Safety**
Liquid waste: acetone, hexanes, EtOAc, and fractions	Acetone, hexanes, ethyl acetate are flammable
Solid waste: pipets, column with silica, dry silica, TLC plates, CAF, ASP, ACE	Caffeine is a *stimulant* and is NOT to be ingested or taken home
Thank you for cleaning your work station: Wash glassware, put away equipment, and wipe benchtops	Silica is an irritant

All used pipets & broken glass go in the glass waste box.

Please do not throw away glass in the trash as it creates an unexpected occupational hazard for our custodial staff.

Thank you for participating in community set up & clean up tasks ☺

Pre-lab Questions / Quiz – see your class notes!

1. Each Excedrin tablet contains 250 mg aspirin (ASP), 250 mg of acetaminophen (ACE), and 65 mg of caffeine (CAF). Calculate the **theoretical percent (%) recovery** of each component using the mass of one tablet (675 mg). Note: there are also inactive ingredients in the tablet.

   (a) ASP **(b)** ACE **(c)** CAF

2. **THE STATIONARY PHASE:** What stationary phase is used in the column and in TLC analysis? Is this substance considered polar or non-polar?

3. **THE MOBILE PHASE:** List each of the solvents / solvent mixtures with ratios used to run the column in order from least to most polar.

4. Go to pubs.acs.org to perform a citation search in the *Journal of Chemical Education*, volume *88*, page 1413 (proper reference format: Revell, K. D. *J. Chem. Ed.* **2011**, 88, 1413). This requires campus access or remote log-in to view the full article. Read this brief article and **report the order that the Excedrin components are expected to elute from the column.**

   - The article is also under Canvas Files > Experiment 1, but it's good practice to try and look it up yourself!

5. Use the *J. Chem. Ed.* Article above and **report the mobile phase for TLC** as well as the **expected R_f values** for each component.

6. **THE SAMPLE:** What **functional groups** do ASP and ACE each contain? Indicate the **intermolecular force (IMF)** associated with *each functional group*: ion-dipole, hydrogen-bonding, dipole-dipole, or dispersion / van der waals forces.

   a. Practice identifying IMFs in the free *Chirality-2* mobile app!

Take the **Canvas Exp 1 pre-lab quiz** *before* your enrolled section – see Canvas for due date

- The quiz incorporates the questions below - the questions may be reworded.
- **Be prepared with your responses to the pre-lab questions *before* starting the quiz.**
- There is a 20-minute time limit on the quiz and you get two attempts.
  - Make sure you have enough time to complete the quiz - you can't save and come back later.
  - If you choose to re-take the quiz, your grade will be the highest of the two attempts.

Though we encourage collaboration in this class, this is an individual quiz.

- The responses should be a product of your original work so that you are assessed on *your* understanding of the material.
- Sharing your quiz or your responses in any format (screenshots, email, CHEGG, social media, text, carrier pigeon, etc.) is in violation of the UCSC academic integrity policy.

LAB REPORT – see your class notes!

No abstract or 'experimental methods' section for the Exp 1 report.

In-lab Questions – numbered responses that incorporate the questions, no need to include the exact question

- Discuss with your partner during lab.
- Work on your own to type your responses in complete sentences for the individual lab report.
- Please **select pages** after uploading to GradeScope.

1. Report all TLC **retention factor (R_f)** values in one table (see recommended format below). **Identify** each spot as CAF, ASP, or ACE by entering the R_f values in the appropriate column for each sample. Account for all spots in each fraction if more than one was present, and indicate which spot was more prevalent (darker), if applicable. Show **R_f sample calculations** for ASP, ACE, and CAF standards.

TLC results for Excedrin column separation (R_f values)

	ASP standard	**ACE standard**	**CAF standard**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
ASP										
ACE										
CAF										

2. Discuss whether the column separation was successful using the TLC results. Report which **fractions were combined and/or concentrated** to obtain ASP, ACE, and CAF. All fractions should be accounted for, including those that were disposed of in the waste without concentration.

3. Report the **mass recovery** of each component after isolation. Calculate the **percent (%) recovery** of each component from the tablet (similar to pre-lab #1). Show your work.

(a) ASP **(b)** ACE **(c)** CAF

4. Comment on your **actual vs. theoretical recoveries**. List specific parts of the procedure where product may have been lost.

5. Explain the **order of separation** of Excedrin components on the column given what you know about functional groups, polarity, and acid-base chemistry (*hint: **hydrogen-bonding** and **ion-dipole interactions** should be included in your response).* Were the **results as expected**?

- For fun - Practice functional group and IMF identification in the free *Chirality-2* mobile app!

6. What role does the **acetic acid** play as part of the **TLC solvent mixture**?