Experiment 2 – Two Base Extraction of Excedrin Components

Learning Objectives

- Understand principles behind acid-base extraction
- Critical analysis of extraction techniques
- Analyze data to assess purity and success of experiment
- Understand the role of functional groups and acidity / basicity on separation
- Predict sources of error and understand their effects on results

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How to Prepare for Lab + Assignments - Follow Canvas Exp 2 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 2 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 2 worksheet** and prepare your **lab notebook**…

Lab Notebook Preparation – *worksheet = detailed 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 2 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: Acid-Base Extraction

The solubility of organic compounds is primarily dependent on polarity. You may recall "*like dissolves like,*" meaning polar compounds dissolve in polar solvents and non-polar compounds dissolve in non-polar solvents. It is safe to assume that most organic compounds of medium to low polarity have limited solubility in water. More polar compounds like alcohols are more likely to be soluble in water, but are only sparingly soluble when there are six or more carbons present in the molecule. In this lab, students will utilize acid-base chemistry to separate a mixture based on preferential solubility in water or ethyl acetate (EtOAc), a polar organic solvent. Excedrin is an over-the-counter analgesic containing the active ingredients aspirin (ASP), caffeine (CAF), and acetaminophen (ACE) that can be separated through **acid-base extraction** (**Figure 1**).

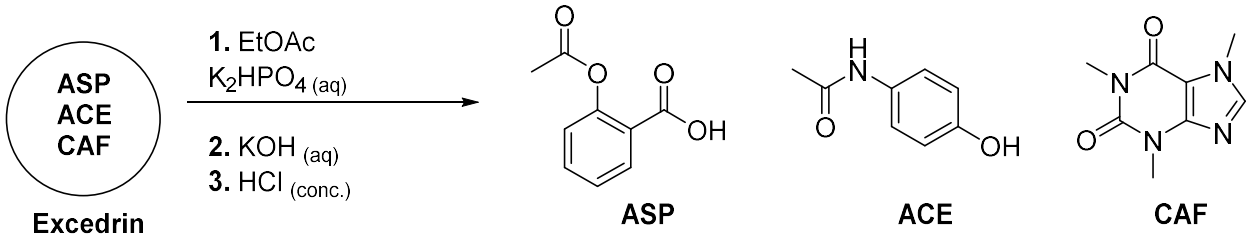

Figure 1. Separation of Excedrin components using acid-base extraction

Acids (HA) react with bases (B) to form a conjugate base (A^-) and a conjugate acid (^+BH) (**Figure 2**). It is likely that one or both of the products are ionic compounds, making them significantly more soluble in water than their non-charged counterparts. In this experiment, we will learn how to take advantage of this change in solubility for the separation of a mixture of acids and bases.

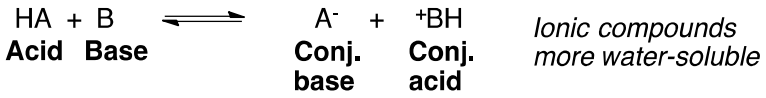

Figure 2. A generic acid-base reaction.

The functional groups of interest in organic acid-base chemistry are strongly acidic carboxylic acids, weakly acidic phenols, and basic amines. Carboxylic acids are deprotonated equally well by weak and strong bases such as dibasic potassium phosphate (K_2HPO_4) and sodium hydroxide (NaOH), respectively. The by-products are different but both reactions form a **sodium carboxylate salt**, which is more water-soluble than the acid (**Figure 3**). In this experiment, the carboxylic acid in ASP is deprotonated with K_2HPO_4 to initiate separation from ACE and CAF. More on this later!

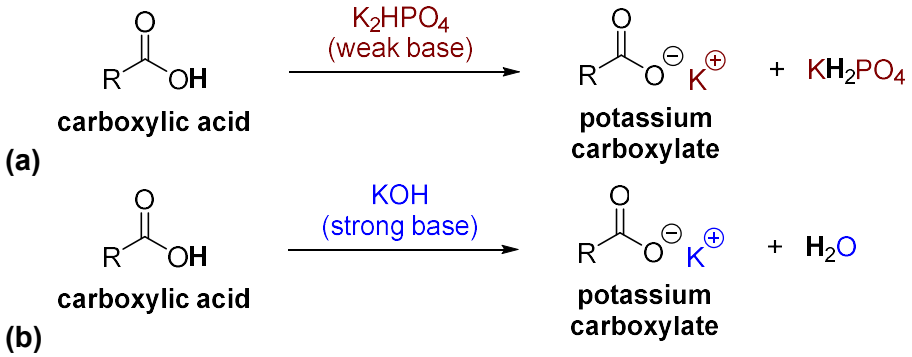

Figure 3. Reaction of carboxylic acids with **(a)** weak base and **(b)** strong base.

Phenols are significantly less acidic than carboxylic acids. Phenols do not react with weak bases. A strong base like potassium hydroxide (KOH) is required for the reaction to occur, resulting in a water-soluble **sodium phenoxide salt** (**Figure 3**). Note that the extraction cannot be started with KOH, as both ASP and ACE would react. Instead, the phenol in ACE is deprotonated with KOH *after* ASP has already been removed and separation from CAF is complete. Keep reading to see how that works!

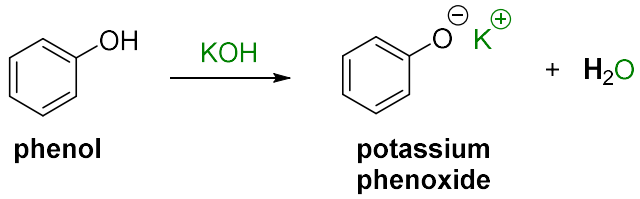

Figure 3. Reaction of phenol with strong base.

Nitrogen-containing organic compounds, also known as alkaloids, tend to be basic. Amines and imines react with strong acids to form water-soluble **ammonium chloride salts** (**Figure 4**). Note that the double-bonded N in the imidazole ring below is more basic and gets the hydrogen instead of the single-bonded N. The latter N is not basic because its lone pair is tied up in resonance and not available to grab the H. This is analogous to the structure of CAF. Though not utilized in this experiment, CAF could be separated from ACE and ASP by treatment with a strong acid.

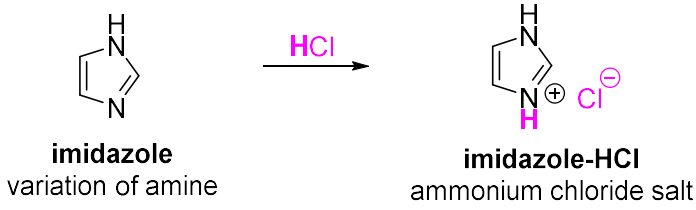

Figure 4. Reaction of imidazole with strong acid.

When both acids and bases are present in a mixture, a liquid-liquid extraction is carried out and at least one of the reactions above is performed. The mixture is dissolved in an organic solvent and a solution of either acid or base is added. The unreacted component is extracted in the organic layer and the reacted component, a salt, is transferred to the aqueous layer.

In this experiment, students will separate a mixture containing a carboxylic acid (ASP), phenol (ACE), and an amine (CAF). The extraction can be started in one of two ways: **(1)** react the carboxylic acid with a weak base or **(2)** react the amine with an acid. Either way will theoretically work, but let's work through the example that starts with a mildly basic extraction (**Figure 5**).

The mixture is dissolved in an appropriate organic solvent, in this case ethyl acetate (EtOAc), and this solution is extracted with a weak base. The organic layer (**ORG**) contains unreacted phenol and amine. The mildly basic aqueous layer (**AQ$_{basic}$**) contains the carboxylate salt (the conjugate base of a carboxylic acid). The carboxylate is reprotonated with acid, thus precipitating from the solution, and permitting isolation *via* filtration. The remaining organic layer is extracted with a strong hydroxide base to deprotonate the phenol, leaving the phenoxide salt (conjugate base of phenol) in the basic aqueous layer and the unreacted amine in the organic layer. The amine can be isolated by drying (ex. Sodium or magnesium sulfate), filtering off the drying agent, then evaporating the solvent in a rotary evaporator (rota-vap). The phenoxide salt must be acidified (reprotonated) and extracted into EtOAc before being dried, filtered, and concentrated.

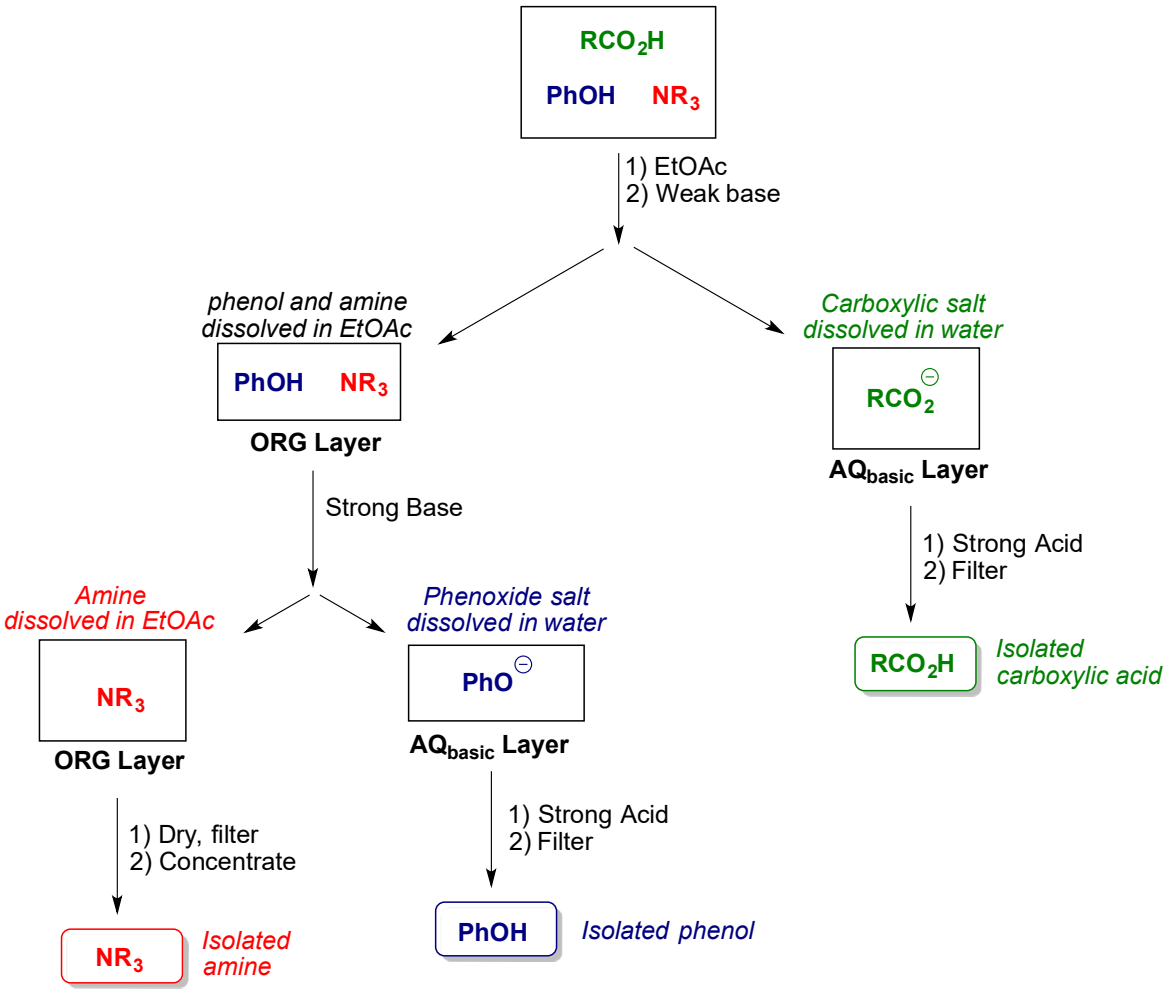

Figure 5. Flow chart for the acid-base extraction of a carboxylic acid from an amine.

A similar procedure is employed in the separation of the three active ingredients in Excedrin. The active components are separated from the inactive ingredients through solid-liquid extraction with EtOAc followed by filtration. Liquid-liquid extraction is used to separate ASP, ACE, and CAF. The solution of active ingredients in EtOAc is treated with $K_2HPO_{4\,(aq)}$, causing two layers to form in the separatory funnel. This weak base reacts with aspirin's carboxylic acid group, causing the extraction of ionized, deprotonated ASP into the **aqueous layer (ASP-AQ)**. This layer is separated from the **organic layer (ORG)**, which contains ACE and CAF as a solution in EtOAc. The **ASP-AQ** layer is treated with HCl to protonate and precipitate ASP from solution. ASP is then isolated by vacuum filtration.

The **ORG** layer is treated with aqueous potassium hydroxide ($KOH_{(aq)}$). This strong base reacts with ACE's phenol, creating phenoxide ions that are extracted into the AQ layer (**ACE-AQ**). This is separated from the **ORG** layer, which now contains only CAF. The **ORG** layer is dried ($MgSO_4$) to remove residual water, then concentrated to isolate solid CAF. The **ACE-AQ** layer is treated with strong acid (HCl) to reprotonate the phenoxide, but unfortunately ACE does not precipitate. Instead, an acidic aqueous extraction is performed. The acidic **ACE-AQ** layer is instead extracted with EtOAc. ACE migrates into the **ORG** layer, which is then dried, filtered, and concentrated to isolate solid ACE.

Keep in mind that in each liquid-liquid extraction, there is no guarantee that 100% of the compounds end up in the expected layer (refer to the pre-lab videos on liquid-liquid extraction). TLC will be used to determine the effectiveness of the separation of each component. IR spectroscopy will be used to confirm the identity of each compound.

PROCEDURE

Procedure Diagrams must be complete in your notebook before you can start the lab – worksheet on Canvas

** **All steps involving ethyl acetate (EtOAc) must be performed in the fume hood.** **

1. Solid-liquid extraction of Excedrin's active ingredients - Obtain the mass of a single tablet of Excedrin® and crush using a mortar and pestle. Add 20 mL of EtOAc to the mortar and mix with a stir rod in the fume hood for 5 minutes. The three active components will dissolve and the starch 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. Collect the filtrate directly in a separatory funnel secured on a support ring on a ring stand.

2a. Extraction with weak base - Add 10 mL of $K_2HPO_{4\,(aq)}$ (aqueous dibasic potassium phosphate) to the separatory funnel. Cap then invert the funnel twice, holding onto the cap. Vent into the fume hood by holding the funnel upside-down and open the stop cock with the tip pointing *away from your face*. Continue to mix and vent frequently for at least 3 minutes. A chemical reaction is taking place and proper time must be given for components to travel to the preferred layer. Drain the mildly basic aqueous layer containing deprotonated aspirin into a labeled scintillation vial (**K_2HPO_4 – ASP AQ**) and set aside. The organic layer will remain in the separatory funnel. Extract the organic layer with an additional 3 mL of K_2HPO_4 (add 3 mL of $K_2HPO_{4(aq)}$, mix and vent for several minutes, then drain into the **K_2HPO_4 – ASP AQ** vial). The organic layer remains in the funnel. One student in the pair should move onto "**Isolation of Aspirin**" using the combined **K_2HPO_4 – ASP AQ** extracts.

2b. Extraction with strong base - Add 10 mL of 1 M KOH to the separatory funnel. Mix the layers for 3 minutes (vent early and often into the fume hood). Drain the aqueous layer containing deprotonated acetaminophen into a second small, labeled container (**KOH – ACE AQ**) and set aside. Extract the organic layer with an additional 3 mL of KOH (add 3 mL of KOH, mix & vent for a few minutes, then drain the aqueous layer into the **KOH – ACE AQ** vial). Keep the organic layer in the funnel.

2c. Isolation of caffeine - Wash the remaining organic layer with 10 mL of *aq*. NaCl (brine); add the brine to the funnel and mix for 1 minute before draining the AQ layer. Separate the layers, draining the organic layer into a small, labeled Erlenmeyer flask. The brine wash (aqueous) should be kept in a separate container labeled "waste" and transferred into the liquid waste at the end experiment. Use an additional 2 mL of EtOAc to rinse any residual **CAF** from the walls of the separatory funnel. Remove any visible water from the bottom of the Erlenmeyer using a pipet. Dry the organic layer by adding two spatula tips of anhydrous sodium sulfate (Na_2SO_4). Allow the capped organic layer to sit with occasional swirling for 5 minutes (move onto one of the isolation steps below while waiting). Decant the organic layer using a small glass funnel with loosely packed cotton into a pre-weighed 50 mL round-bottom flask (RBF). Concentrate the dried organic extracts using a rotary evaporator (rota-vap). This concentrated **CAF** extract may either be a liquid or solid, depending on purity. Obtain the mass of caffeine by difference with the original flask then transfer into a labeled vial.

2d. Isolation of Aspirin – This step may be performed on the benchtop. Tear one 2-inch piece of pH paper into many small squares to conserve. Determine the pH of the **K_2HPO_4 – ASP AQ** solution by dipping a stir rod into the solution then touching to a small piece of pH paper on a watch glass. Obtain 10 mL of 6 M HCl in a labeled test tube. Slowly add 6 M HCl drop-wise to the **ASP AQ** solution, swirling and taking pH readings after every 5-10 drops, until the solution is acidic (pH 2 or less). Do not rush this process! Re-label the vial "**Acidic ASP AQ.**" It may be necessary to get additional 10 mL portions of HCl. Please conserve and take only small amounts at a time. A significant amount of ASP should precipitate, creating an opaque solution. Collect the product by vacuum filtration. Allow the solid to dry with the vacuum on for 10-15 minutes. Obtain the mass of the solid then transfer into a capped vial labeled "**ASP + (initials)**."

2e. Acidic extraction and isolation of acetaminophen – Carry out the same acidification procedure used to isolate aspirin (add 6M HCl, take pH to 2 or less). Label the vial "**Acidic ACE AQ**." The neutral protonated compound acetaminophen is in the aqueous solution and will be extracted with EtOAc. Transfer the acidic aqueous solution to the separatory funnel and add 15 mL of EtOAc. Mix and vent for 3 minutes, then drain the aqueous and organic layers into separate flasks. Extract the aqueous layer with an additional 15 mL of EtOAc (add 15 mL EtOAc to the aqueous layer, mix & vent for several minutes, then remove the aqueous layer). Wash the combined organic extracts with 10 mL of brine.

Separate the layers and dry the organic layer over anhydrous Na_2SO_4 for 5 minutes (remove visible water from the organic layer by pipet, add the drying agent, and allow to sit with occasional swirling). Filter into a pre-weighed 50-mL RBF then concentrate using a rota-vap. The concentrated extracts may either be a liquid or solid, depending on purity. Obtain the mass of **ACE** by difference, transfer to a labeled vial, and proceed to analysis.

ANALYSIS PROCEDURE

3a. TLC - TLC standard R_f values were obtained in Exp 1 and can be referred to without repeating this part of the experiment. Dilute a small amount (microspatula tip) of each component isolated in this experiment with 1 mL of acetone in a test tube. Analyze by TLC using 1:2 hexanes / ethyl acetate with 1% acetic acid as the mobile phase. Visualize the plates under a UV lamp, circle the spots, and calculate all R_f values. Repeat as necessary to obtain optimal results (Ex. if spots are too large / smeared - dilute your samples; if lanes are slanted - be more careful when placing the plate in the jar and do not move the jar).

3b. IR – After TLC analysis, determine whether each of the isolated components are pure (1 spot). Do not attempt to take an IR spectrum of contaminated samples. Instead, take the IR of a standard. Compare your predicted IR spectra tables with labmates – instructions for predicting spectra are in the pre-lab questions. Obtain the IR of each pure compound using a Nujol mull (grind the mull for at least one minute). Identify any peaks within the expected ranges based on the functional groups and bonds within ASP, ACE, and CAF.

Table 1. Clean-up and Safety

Clean-up – leave the lab as you found it!	**Safety**
Glass waste: uncontaminated pipets only	HCl and KOH are *corrosive & toxic*.
Liquid waste: contents of rota-vap trap, TLC solutions	Acetone and ethyl acetate are *flammable*. Caffeine is a *stimulant* and is NOT to be ingested or taken home.
Solid waste: filter paper, used pipets	
Product waste bag: product vials	Do not look directly into the UV lamp.
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 ☺	

References & Supplemental Reading

Mohrig 4th ed. Chapter 10.1-10.5 (Extraction), Drying agents (Chapter 11), TLC (Chapter 18)

Revell, K. D. *J. Chem. Ed.* **2011**, 88, 1413.

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

Take the **Exp 2 pre-lab quiz** *before* your enrolled section – see due date on Canvas

- 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.

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1. Classify ACE, ASP, and CAF as **acidic, basic, or neutral**. Indicate which **functional group** determines the acid-base properties of each.

2. What **reaction** takes place in the addition of **dibasic potassium phosphate (K_2HPO_4)** to the mixture of ASP, ACE, and CAF? Show the **chemical equation** with full structures in support of your answer. Indicate whether each component (ASP, ACE, and CAF) should be in the **aqueous** or **organic layer** after the reaction.

3. What **reaction** takes place in the addition of **potassium hydroxide (KOH)** to the organic layer (after pre-lab #2)? Show the **chemical equation** with full structures in support of your answer. Indicate in which layer (**AQ or ORG**) each component should be after the reaction.

4. What **volume of 6 M HCl** is required to react with (neutralize) **13 mL of 1 M K_2HPO_4**? …to neutralize **13 mL of 1 M KOH**? Show your work. Hint: $M_1V_1 = M_2V_2$

5. Predict the IR spectra of ASP, ACE, and CAF using the tables in the **Exp 2 Worksheet** and the steps below.

- Identify each functional group (FG) in ASP, ACE, and CAF.
- Use the **IR Tables** on Canvas to find the IR active bonds within each functional group (FG) and its expected wavenumber range.
    - **List all bonds and vibrations** for each FG, as there may be multiple.
        - Some bonds have two different vibrations (ex. C-H bonds in arenes stretch and bend).
    - Determine if double bonds are **saturated or conjugated** (resonance with another pi bond).
- The substitution patterns of the arene ring affect the **C-H bending** vibrations. Use **IR Table 2** to determine the specific range of C-H bending frequencies.

LAB REPORT

Canvas > **Experiment 2 Report** for submission details

Upload to GradeScope (GS) after both parts of the lab – see due date on Canvas

- **Select Pages** to correlate your responses to the GS outline ☺

In-Lab Questions – see your class notes! Discuss with your partner during lab. Many of these questions are included in the Exp 2 Worksheet. Type your responses in complete sentences for the lab report, leaving space to draw by hand where necessary.

1. Report the **mass recoveries of ACE, ASP, and CAF** after isolation. Calculate the **% recovery** of each component from the initial amount of Excedrin used. Show your work.

2. Compare the recoveries above to the theoretical recoveries (Exp 1, pre-lab #1) and list the specific parts of the procedure **where product may have been lost**.

3. Report and discuss the **TLC results**: Make a table with the **R_f values** for each spot in each sample (4 columns – sample, ACE, ASP, CAF). **Identify** each spot as ACE, ASP, or CAF by entering the R_f value in the appropriate column for each sample. Include standard R_f values from Exp 1. **Explain** whether or not the separation was effective.

4. Interpret the **IR spectra** of ACE, ASP, and CAF. Type all 3 completed IR tables from the **Exp 2 Worksheet** (no hand-written tables in the results section please). Use three sentences (one per compound) **to describe** how the IR spectra can be used to positively identify each individual compound (unique stretches in each).

5. Compare the results of Excedrin separation *via* column chromatography (Exp 1) and acid-base extraction (Exp 2) as follows. While one method may not be generally *better* than the other, there should be some differences in the points below.

(a) Restate the **recoveries** of each component by each method.

(b) Which method produced **greater amounts** of ASP, ACE, and CAF? Note that different methods could be more ideal for isolating different components.

(c) Which method yielded **higher purity** of each component as determined by TLC?

(d) Based on your results and discussion above, was **column chromatography or acid-base extraction** more effective for separation of Excedrin components?