CHEM 213 Technique Experiments

Experiment 4: Thin Layer Chromatography

<table>
<thead>
<tr>
<th>Number of labs</th>
<th>one</th>
<th>Reactions performed</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals used:</strong></td>
<td>Ibuprofen, aspirin, caffeine, acetaminophen, phenacetin, solvents</td>
<td><strong>Supplies needed:</strong></td>
<td>Large beakers, TLC spotting capillaries, TLC plates, foil</td>
</tr>
<tr>
<td><strong>Techniques:</strong></td>
<td>thin layer chromatography</td>
<td></td>
<td></td>
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</tbody>
</table>

Prelab

*Reading Assignment: Laboratory Techniques in Organic Chemistry, 4th Ed.*


Prepare a brief experimental plan in your notebook, and an overview of the problem to be solved.

*Safety*

*Goggles required! Spotting capillaries are easily broken, small glass particles can cause cuts.*

*Scenario:* A patient who swallowed a large number of drug tablets was just wheeled into the emergency room of a local health clinic suffering from a drug overdose. The bottle that contained the drug is missing but some unused tablets were left on a counter in the bathroom. The patient’s spouse recalls seeing a bottle of over-the-counter analgesic drug in the medicine chest but doesn’t remember what was on the label. Because there was no nearby toxicology lab in operation, the clinic rushed the tablets to your supervisor for analysis. Your assignment is to determine the identity of the appropriate treatment.

*Over-the-counter pharmaceuticals:*

Druggists were once called chemists; in Great Britain, they still are. Whenever you go to the drugstore to buy a bottle of aspirin, Advil, Tylenol, or another of the dozens of different analgesic (painkilling) drug preparations that are available, you are purchasing an organic chemical or a mixture of several chemicals. That shouldn’t be surprising, since all matter—including you—is composed of chemicals, where chemical is just another name for a substance (element or compound). Most of the materials we encounter in daily life are complex mixtures of substances, whereas many drugs are reasonably pure substances or mixtures of only a few substances. In fact, most analgesic drug preparations contain only one or two active ingredients: aspirin, acetaminophen, ibuprofen, or some combination of these. The most popular combination is aspirin and acetaminophen, but salicylamide (a chemical relative of aspirin) is combined with aspirin in a few drug preparations. Caffeine is sometimes added to an analgesic preparation for its stimulant effect and because it interacts with some analgesics to enhance their pain-relieving effects.
A small amount of starch is generally added as a binder to hold the tablets together. Table 1 shows the composition (not including the starch) of some representative analgesic preparations.

Most analgesic drugs do more than just kill pain. Aspirin, acetaminophen, and ibuprofen are also antipyretics, meaning that they reduce fever. Aspirin and ibuprofen are both nonsteroidal anti-inflammatory drugs (NSAIDS), meaning that they help reduce swelling and other symptoms of inflammation. Aspirin, in fact, has such a surprisingly wide range of benefits that it can be regarded as a true “wonder drug”. In recent years, it has been shown to reduce the incidence of heart disease, strokes, and certain cancers. It may help prevent cataracts, reduce the occurrence of gallstones, and improve brain function in people who have suffered small strokes.

Although aspirin (acetylsalicylic acid) is the most widely used drug in the world, nobody understood how it worked until 1971, when the British pharmacologist John Vane showed that aspirin blocks the overproduction of natural biological substances called prostaglandins by deactivating a key enzyme required to manufacture them, prostaglandin synthase. Although prostaglandins are an essential biological regulators, and oversupply of certain prostaglandins can promote the formation of blood clots that lead to heart attacks or strokes, while others trigger pain, fever, and inflammation.
Table 1: Composition of some analgesic drug preparations (in milligrams per tablet)

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Aspirin</th>
<th>Acetaminophen</th>
<th>Ibuprofen</th>
<th>Salicylamide</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Anacin</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Aspirin*</td>
<td>325</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.C. Tablets</td>
<td>325</td>
<td>250</td>
<td>95</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Excedrin</td>
<td>250</td>
<td>250</td>
<td></td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Tylenol</td>
<td></td>
<td>325</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*5-grain tablet (1 grain = 64.8 mg)

Recent studies have shown that prostaglandin synthase functions like a factory assembly line: raw materials enter one end of a channel that passes through the enzyme and leaves the other end as fully assembled prostaglandin molecules. Aspirin molecules sabotage this process by blocking the channel, thereby preventing raw materials from getting by. Aspirin appears to inhibit cancers of the digestive system by a different mechanism, stimulating the production of cancer fighting substances used by the body’s immune system.

If the other analgesics can’t compete with aspirin in versatility, they do have certain advantages over aspirin. Aspirin tends to promote bleeding, especially in the stomach. And it has been implicated in Reye’s syndrome, a rare but often fatal disease that affects children. Acetaminophen is just effective as aspirin at reducing pain—but overdoses of acetaminophen can cause serious liver damage, especially when taken in conjugation with alcohol. Ibuprofen is a powerful analgesic with approximately the same painkilling effect as aspirin at one-third the dosage. It is especially effective in treating arthritis and menstrual cramps, but it can cause problems in people with asthma, ulcers, high blood pressure and kidney, liver or heart disease.

In this experiment, you will use TLC to identify the components of the analgesic drug that your instructor assigns. Your drug may contain from one to three of these substances: acetaminophen, aspirin, caffeine, ibuprofen, and salicylamide. From the array of components you discover by TLC, you will be able to identify the OTC Drug Name from Table 1.

You will prepare a solution of the drug by dissolving part of a tablet in 1:1 ethanol / CH₂Cl₂, then spotting a TLC plate with this solution along with standard solutions of all the active substances the drug tablet is likely to contain.

The TLC plate can be developed with a solvent mixture such as ethyl acetate / acetic acid (200:1). By using two different methods to visualize the spots, and comparing the Rf values to your unknown’s spot(s) with those of the standards, you should be able to match each unknown spot with the spot of a known substance. Then you will refer to Table 1 above to find out which commercial drug preparation contains the components you have identified.

Caffeine is only a minor component of a drug such as Excedrin, so its spot might be too faint to see clearly in some cases. In this event, you can usually arrive at a correct commercial drug by comparing the remaining components with those in the tablet. For example, Excedrin is the only drug listed that contains both aspirin and acetaminophen, and B.C. is the only one that contains salicylamide.
**Procedure:**

*Preparation of the developing chamber*
- Unless your instructor indicates otherwise, use ethyl acetate / acetic acid (200:1) as the developing solvent.
- Fill an appropriate developing chamber (see CH. 18) containing a paper wick to a depth of about 5 mm with the solvent.
- Cover it with foil or an appropriate lid, and slosh the solvent up the sides of the chamber to moisten the wick. Then set it aside under a hood while you prepare the TLC plate.

*Preparation and Development of the TLC plate*
- Obtain a quarter tablet of the unknown analgesic drug, and grind it to a powder with a flat-bottomed stirring rod or place it between weighing papers and crush it with the bottom of a beaker. (If the tablet is coated, remove as much of the coating as you can before grinding.)
- Transfer the powder to a test tube, and add 2.5 mL of 1:1 ethanol / dichloromethane. Thoroughly mix the solid in the solvent with a stirring rod to dissolve as much of the solid as possible.
- Use a syringe pipette to transfer the solution to a small vial, leaving the solid behind, and cap the vial.
- Spot the unknown solution and all of the standard solutions provided on a silica gel TLC plate that has a fluorescent indicator, and label the spots.
- To avoid cross-contamination, use a different micropipette (or other spotting device) for each solution. You may share spotting capillaries for the same standard solutions. If possible, the unknown should be spotted in different concentrations (from 1-3 applications) at two or more locations along the starting line.
- Develop the plate *under the hood* in the developing chamber you prepared. Do not disturb the developing chamber until the plate is developed. Mark the solvent front before the plate dries with a pencil.

*Visualization and Analysis*
- When the TLC plate is dry, observe the spots under short-wavelength (254 nm) ultraviolet light, outline them with a pencil, and mark the center of greatest intensity for each spot. (Take care! Do not directly at the light source.)
- Calculate the R_f value of each spot, and identify the active ingredient of your unknown by comparison with the R_f values and visualization results for the known spots. Use Table 1 to determine the commercial name of your analgesic drug preparation. (Your tablet may be a generic equivalent of one of those listed.) Turn in your TLC plate with your report.

*Waste Disposal:* Leftover eluting solvent should go in waste container (non-halogenated). Spotting solutions on the well plates should be rinsed in the sink and the well-plates thoroughly cleaned and returned. All capillaries should go in glass garbage.