Thin Layer Chromatography (rev 3/2020)

Thin layer chromatography (TLC) is a method for analyzing mixtures by separating the compounds in the mixture based on polarity. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique—microgram (0.000001 g) quantities can be analyzed by TLC—and it takes very little time for analysis (about 5-10 minutes).

TLC consists of three steps: spotting, development, and visualization. Photographs of each step are shown on the course website. First, the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute (about 1%) solution. Spotting consists of using a micro pipet to transfer a small amount of this dilute solution to one end of a TLC plate—in this case, a thin layer of powdered silica gel that has been coated onto a plastic sheet. The spotting solvent quickly evaporates, leaving behind a small spot of the material.

Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent (eluent), which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate. The outcome depends upon a balance among three polarities—that of the plate, the development solvent, and the spotted material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has traveled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate.

Visualization of colored compounds is simple – the spots can be directly observed after development. Because most organic compounds are colorless, one must employ an alternate method of visualization. The silica gel manufacturer coats the silica gel on the TLC plate with a material that fluoresces (illuminates) green under ultraviolet (UV) light (more specifically 254 nm light). A spot will quench the fluorescence and appear as a dark spot on a glowing background. *Not all compounds quench the material. Ones that do are said to be "UV-active" and ones that do not are "UV-inactive."* To be UV-active, compounds must possess a certain degree of conjugation, which occurs most commonly in aromatic compounds. One can then outline the spots with a pencil, while under the UV light, to mark their location. Staining the spot by chemical means is a second method of visualization. Many stains are possible to use, but the easiest is iodine, $I_2(s)$. The TLC plate is placed into a chamber contain $I_2(s)$, which sublimes and fills the chamber for a few minutes with iodine vapor. Most organic compounds will form a dark-colored complex with iodine. It is good practice to use at least two visualization techniques in case a compound does not show up with one particular method, i.e., if the compound is UV-inactive.

The R_f value, or **R**etention **F**actor, is used to quantify the movement of the materials along the plate and is a dimensionless quantity. R_f is equal to the distance traveled by the substance divided by the distance traveled by the solvent. Its value is always between zero and one. A TLC analysis might be summarized by something like, "Using a silica gel plate and ethyl acetate as the development solvent,

unknown mixture X showed three spots having R_f's of 0.12, 0.25, and 0.87. Comparing these R_f's with the R_f's of known compounds might enable a tentative identification to be made. Note that observing three spots means only that there are at least three components in the mixture. Some components may have such similar polarities that they appear under one spot after development.
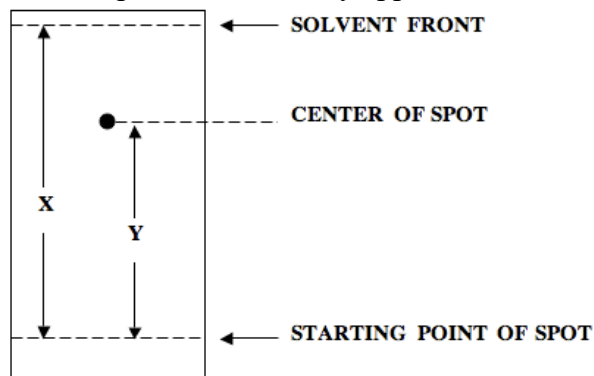

$R_f = Y/X$ (always ≤ 1)

If a development solvent of too high a polarity is used, all components in the mixture will move along with the solvent and no separation will be observed (R_f's will be too large). If the solvent is of too low a polarity the components will not move enough, and again separation will not occur (R_f's will be too small). In practice, different solvents or mixtures of solvents are tried until a good separation is observed. Typically an effective solvent is one that gives R_f's in the range of 0.3 - 0.7.

Note that the spotting solvent is simply used as a vehicle to transfer the material to be analyzed to the TLC plate. Once the transfer is made, the spotting solvent evaporates. It has no effect on the separation. It is the development solvent that effects the separation.
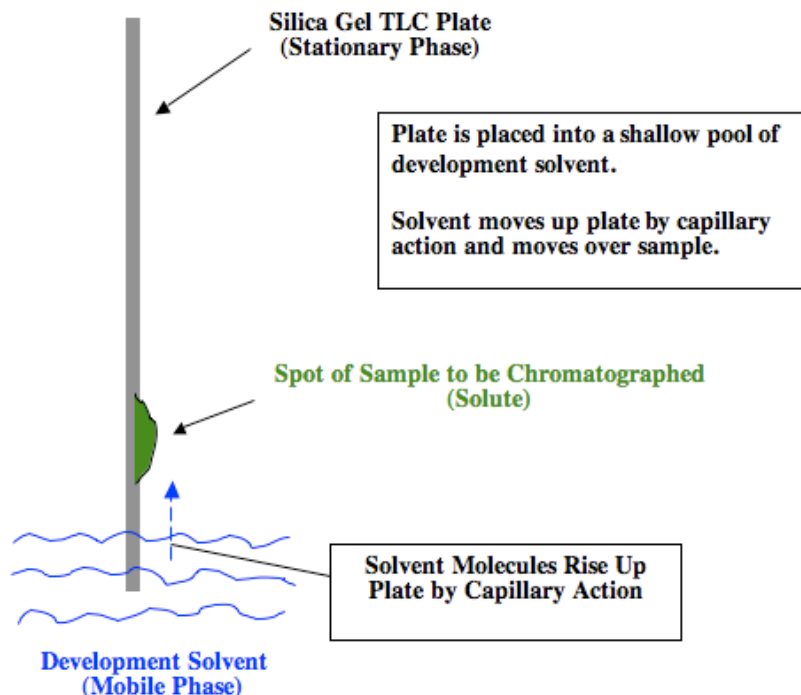
What's going on at the molecular level during development? There are three components in TLC: (1) the TLC plate (stationary phase), the development solvent (mobile phase), and the sample to be analyzed (solute). In our experiment, the TLC plate consists of a thin plastic sheet coated with a thin layer of silica gel, a portion of the structure of which is shown below.
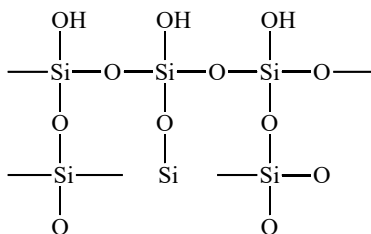

Silica gel consists of a three-dimensional network of thousands of alternating silicon and oxygen bonds, with O-H groups on the outside surface. Silica gel is simply very finely ground very pure sand. It should be noted that silica gel is highly polar and is capable of hydrogen bonding.

Consider the side-on view of the development of a TLC plate below. As the solvent travels up the plate over the compound(s) to be analyzed, an equilibrium is set up between the solute wanting to be in the solvent and wanting to be held onto the silica. The silica gel binds to the solute and the

development solvent tries to dissolve it away, carrying the solute(s) along as the solvent travels up the plate.

Silica Gel TLC Plate (Stationary Phase)

Plate is placed into a shallow pool of development solvent.

Solvent moves up plate by capillary action and moves over sample.

Spot of Sample to be Chromatographed (Solute)

Solvent Molecules Rise Up Plate by Capillary Action

Development Solvent (Mobile Phase)

A balance of intermolecular forces determines the position of equilibrium and thus the ability of the solvent to move the solute up the plate. In other words, would the spot prefer to be stuck on the plate or would it prefer to move along with solvent?
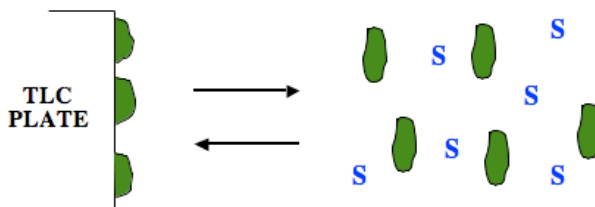

The balance (equilibrium) depends upon (1) the polarity of the TLC plate (constant and high), (2) the polarity of the development solvent (can be varied by using different solvents), and (3) the polarity of the compound(s) in the spot (this varies depending upon what compound(s) is/are in the spot). For example, if a sample consists of two components with unequal polarity, the more polar will tend to stick more tightly to the plate and the less polar will tend to move along more freely with the solvent. Using a more polar development solvent would cause both to move along further. If the approximate structures of the solutes are known, it is possible to make an educated guess as to what solvent or mixture of solvents to use. In practice, however, for a given mixture of compounds to be analyzed, a solvent or mixture of solvents is chosen by trial and error to give the best separation. (A *caveat*: the polarity argument is helpful in understanding the principles of TLC. Because most compounds have some associated polarity, the argument works well. For compounds having very low polarity, however, a lower-polarity solvent may be more effective in moving the solute up the plate.)

To illustrate a TLC experiment, consider the following example of the analysis of a two-component mixture.

TO START, PLACE BOTTOM OF SPOTTED PLATE INTO DEVELOPMENT SOLVENT. ALLOW SOLVENT TO TRAVEL UP PLATE.

AFTER SOME TIME, SOLVENT HAS TRAVELED UP OVER THE SPOT.

LESS POLAR COMPONENT HAS TRAVELED FURTHER.

MORE POLAR COMPONENT HAS TRAVELED LESS FAR.

Table 1. Functional groups in order of increasing polarity.

| |
|---|
| Alkanes |
| Alkyl Halides |
| Alkenes |
| Aromatic hydrocarbons |
| Ethers |
| Esters |
| Ketones |
| Aldehydes |
| Amines |
| Alcohols |
| Carboxylic Acids |

Note that many molecules contain multiple functional groups and that all of the groups would determine the overall polarity.
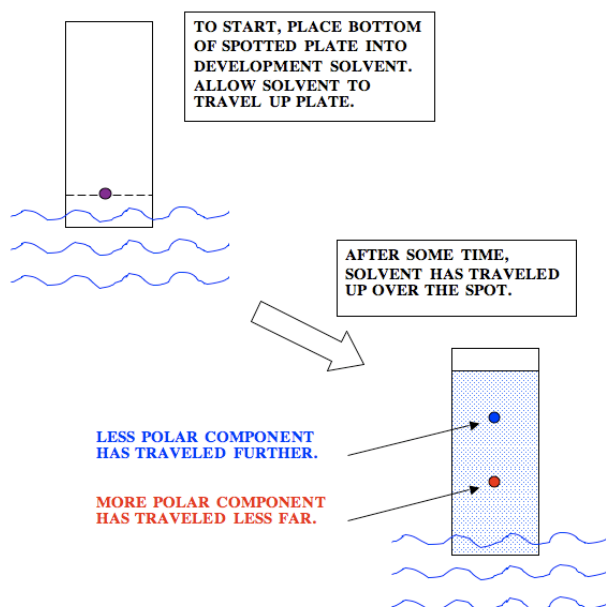
Analgesic Background

Analgesics are any compound/drug that provides relief from pain. They may be prescribed or over the counter (OTC), but more often than not, analgesics are non-addictive, non-habit forming, and therefore readily available to us as consumers. The table below lists some common analgesics with both their generic and brand names. Their chemical structures are also shown.

Analgesic (generic name)	Brand Name(s)
Aspirin	Bayer®
Ibuprofen	Motrin®, Advil®
Naproxen	Aleve®
acetaminophen	Tylenol®

aspirin   ibuprofen   naproxen   acetaminophen
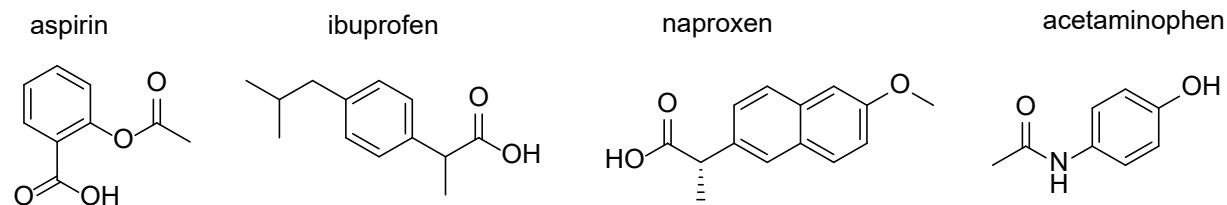
In many instances, analgesics (as well as narcotic, e.g. hydrocodone, pain relievers) are more effective at relieving pain when paired with other substances (known as a synergistic effect). For example, Vicodin® is a mixture of hydrocodone and acetaminophen simply because they are more effective when used in conjunction. Along these lines, some analgesics are sometimes used in combination with caffeine.

Caffeine is a neuro stimulator and also causes the blood vessels to constrict, which restricts the flow of blood. This is known as a vasoconstrictor. For example, when caffeine is paired with aspirin and

acetaminophen, the analgesic properties are increased by nearly 40% (this is now common knowledge and as such DOES NOT require citation). You should look up the structure of caffeine and note it in in your prelab exercise. Again, common knowledge applies.

Prelab: You may either print out your prelab and bring it with you to lab, or bring your computer. Your TA will grade it on the spot before you begin the experiment. For the in lab observations, you may use scratch paper and record later in your ELN, or bring your computer and record directly in your ELN.

Postlab Report: Make sure to use the non-formal postlab report template on the course website!

Experimental Procedure

This experiment consists of two parts: (1) analyzing two different commercially available analgesics to determine the number of organic compounds in each and the identity of those compounds by comparing with known substances; and (2) studying the effect of solvent polarity on R_f. Look over the photos showing TLC on the course website.

The known substances used in part 1 are:
- Aspirin (ASP)
- Acetaminophen (ACET)
- We will also be looking for caffeine (CAFF), if present, in the unknown commercially available analgesics.

### Part 1: Analysis of analgesics
a. Unknown Sample Preparation
   a. Choose two unknowns. Make an approximately 1% solution of each unknown analgesic. To do this, take a small amount (very tip of micro spatula) of the pre-crushed tablets and add it to your 1 dram vial. Add several drops of ethanol. *Commercially available analgesics contain organic solvent insoluble binders, so not all of it will dissolve.* Label your unknowns.
   b. Solutions of the three known compounds will already be made, located on the supply bench.

b. Preparing and Spotting the TLC Plate
   a. The powdered side of the silica gel plate (not the shiny, plastic side) is the side that is spotted.
   b. <u>Lightly</u> draw a pencil line about 1 cm from the end of a plate. Use a pencil, not a pen. Be careful not to scratch the surface of the plate too deeply. Mark three spots along this line indicating where you will spot your samples. <u>The size of the plates means that only three spots can be run on each plate.</u>
   c. Label and spot the samples as follows (see figure below):
      i. Plate 1: 2 of the 3 known compounds, aspirin and acetaminophen (references), plus the first unknown analgesic (unknown 1) will be spotted.
      ii. Plate 2: the same 2 knowns as plate 1, plus the second analgesic (unknown 2).
      iii. Plate 3: Both unknown analgesic 1 and analgesic 2 along with the third known compound (caffeine). (If the plates were wider, you could analyze each unknown on just one plate.)
      iv. To apply spots, dip the microcapillary into the sample. The sample will be pulled up via capillary action. Make each spot as small as possible (less than about 1 mm diameter). To do this, do not hold the spotter to the plate. Simply tap and immediately release. Tap

and release several times to concentrate your sample in a small area. After applying the spots check them under the UV lamp to see that they are of a reasonable size and concentration. The UV light must be held close to the plate to see the spots (CAUTION: do not look directly into the UV light).

    v. If the spot is much too large, prepare a new plate. If a spot is too small or faint, spot again.

    vi. Rinse the spotter with acetone between each use. Do this by having a vial containing a small amount of acetone nearby. Dip spotter in acetone, lightly press against a piece of paper towel, and allow capillary action to withdraw all solvent. Do this 2-3 times to be sure the spotter is thoroughly rinsed.

    vii. To obtain a sample of a known solution, bring a micropipet to the sample solution at the bench, dip it into the solution to fill it, then take it back to your workspace for spotting (Use only micropipets for obtaining samples; do not use Pasteur pipets or MP capillaries to take larger amounts of samples – that could lead to contamination).

    viii. Try to minimize traffic at the center bench and take care to not contaminate the solutions with a micropipet that has already been used.
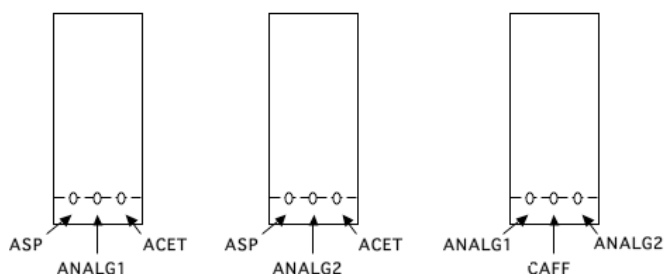
Abbreviations:
ASP – Aspirin
ACET – Acetaminophen
CAFF - Caffeine
ANALG1 – Unknown analgesic 1
ANALG2 - Unknown analgesic 2

c. Development
  a. The development chamber is a small screw-capped jar with a 5.5 cm filter paper placed inside to ensure atmospheric vapor saturation. Each run is relatively brief, so please use only one ethyl acetate development chamber, otherwise there may not be enough for everyone.
  b. Place about 4 mL of ethyl acetate into the chamber (2 mL is a full squeeze of the bulb using a Pasteur pipet) and bring it back to your work area.
  c. Work in the hood. Place the TLC plate using your tweezers into the jar with the spotted side at the bottom. Replace the cap. Keep the cap on the jar at all times except when inserting the plate into or taking the plate out of the jar.
  d. Watch out for the following:
    i. the solvent must be below the level of the spots or the spotted material will dissolve in the development solvent;
    ii. the plate must stand vertically in the jar and the silica gel must not touch the filter paper; the solvent must not be allowed to run all of the way to the top of the plate;
    iii. the correct amount of material must be spotted (checking under UV light before development may help—if spots are too large, another plate can be made; if the spots are too small, more material can be spotted).
  e. After the solvent has almost reached the top of the plate, the plate is removed and the position of the solvent front is marked by pencil **before the solvent evaporates**. Always allow the developing solvent to run almost completely to the top of the plate. This will use the entire plate and allow for the best possible separation.

    f.   The plate is then allowed to dry in the hood. The development takes only a little time so if a plate comes out poorly, another can easily be done.

d. Visualization
    a.   This is done by two methods. First, by short wavelength UV light. (CAUTION: do not look directly into the light.) The light must be held close to the plate to see the spots. Some spots may be very faint. The observed spots should be outlined with a pencil.
    b.   The plate is then placed for a few minutes into a jar containing iodine crystals. Keep the iodine jar capped and in the fume hood. The spots may appear different from those shown with the UV light. They should also be circled.
    c.   A comparison of the two visualization techniques can be made and may help in identifying the unknown spots.
    d.   The iodine method must be done after the UV method because the compound may react with the iodine, possibly changing the results.

e. Calculate the Rf values for the known compounds and for all components of the unknown analgesic. From your results, identify which components are present in a particular analgesic. More than one of the knowns may be contained in your unknown. Some of the components may have very similar Rf's under these conditions. However, their spots may look different upon treatment with iodine, allowing a distinction to be made. Note that caffeine is present in small amounts and may not show up well. Purposely overloading a spot may help to show a component present in small amounts.

IMPORTANT: Do not keep the TLC plates, as the silica will flake off over time and end up at the bottom of your bag. Instead, take one picture of your best plates together and turn it in with your report. Make sure your labels are clear and easy enough to read.

Part 2: **Solvent Effect on R$_f$ Values**
   a.   Three known compounds, anthracene, benzil, and triphenylmethanol, will be developed in two different solvents.
    b.   Prepare two identical plates, spotted with each of those compounds.
    c.   Develop one in ethyl acetate and the other in a mixture of 95% hexane and 5% t-butyl methyl ether (you may use a second chamber for this solvent mixture).
    d.   Calculate R$_f$ values, and in the post-lab write-up, discuss the effect of solvent on R$_f$, keeping in mind the structures and polarities of the compounds and solvents.
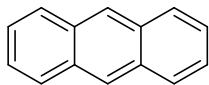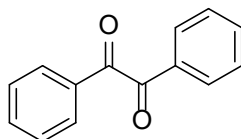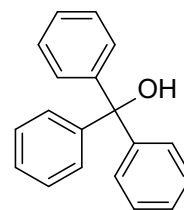
anthracene   benzil   triphenylmethanol

Data Analysis: Place your properly labelled TLC plates on the lab bench and take a picture. Review the picture to be certain it is clear and readable. Keep this photo, and turn it in with your post-lab

report and use it for your data analysis and Results and Discussion section. Place the TLC plates in the solid organic waste container.
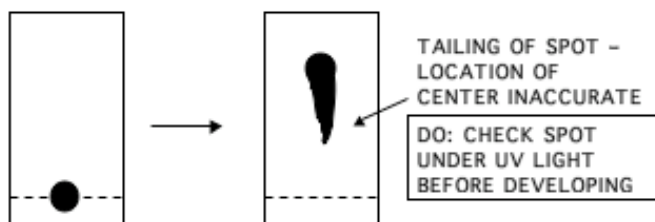
SAFETY: Carefully note the warning about glass slivers above. Do not stare into the UV lamp.

BEFORE YOU LEAVE THE LAB: turn off any electrical equipment that you may have used, put away your equipment, clean up your work areas, close the fume hood sash completely, and ask your TA for her or his signature. In general, please try to keep the lab in as good condition as you found it. If you see caps off of bottles, replace the caps. If you see spilled chemicals, clean them up or at least report it to your TA.

WASTE: Never dispose of glass waste in the regular trash. The custodian could become injured. Dispose of all glass waste in the cardboard "Glass Only" boxes. Used capillary pipets may be placed into these boxes or into the used capillary dishes placed around the lab. Dispose of all solutions and developing solvents in the ORGANIC LIQUID WASTE container in the hood. When finished, pour as much of the solvent as possible into the waste container and leave the screw-capped jars open in the hood so they will dry. Leave the filter paper in the jars.
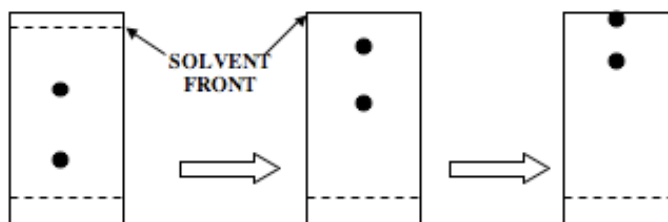

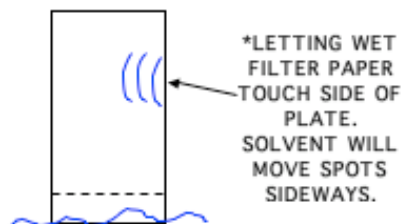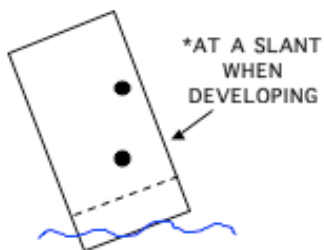THINGS TO WATCH OUT FOR.

* OVERLOADING THE SPOT (SPOT TOO LARGE)

TAILING OF SPOT –
LOCATION OF
CENTER INACCURATE

DO: CHECK SPOT
UNDER UV LIGHT
BEFORE DEVELOPING

* SPOT TOO SMALL – DIFFICULT TO SEE

* ALLOWING SOLVENT FRONT TO REACH TOP OF PLATE

SOLVENT FRONT

IF THE SOLVENT FRONT CANNOT BE SEEN, THE R_f CANNOT BE CALCULATED. STOP BEFORE FRONT REACHES TOP OF PLATE.

*AT A SLANT
WHEN
DEVELOPING

*LETTING WET
FILTER PAPER
TOUCH SIDE OF
PLATE.
SOLVENT WILL
MOVE SPOTS
SIDEWAYS.

Postlab Questions

1.) Arrange the following compounds in order of increasing R_f in a TLC analysis: benzoic acid, benzaldehyde, 3-heptanone, nonane, and cyclohexanol.

2.) Why must the spot be applied to the TLC plate above the level of development solvent?

3.) What will be the result of adding too much sample to the TLC plate?

4.) In a TLC analysis of analgesics, what would be the result if a solvent of too low polarity is used to develop the plate?

5.) The R_f of ibuprofen was found to be 0.32 when neat t-butyl methyl ether was used as the development solvent. What effect would there be on the R_f of ibuprofen if acetone had been used to develop the TLC plate?