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Experiment 4: Chromatography

Parts A & B Thin Layer Chromatography (TLC)

Introduction (Parts A & B)

The objective of this experiment was to identify the specific combination of 2 out of 4 possible compounds using Thin Layer Chromatography.

TLC is a very useful method for identifying compounds, as it is able to separate compounds according to their polarities. This makes it very useful for compound identification, such as the identification of different compounds in pharmaceuticals.

Scientific Principles (TLC)

1. Consider a TLC analysis where the mobile phase is a 20:80 ethyl acetate/petroleum ether solution, the stationary phase is silica gel, and the spotted mixture contains two compounds (one is polar; the other is nonpolar), give the expected outcome and explain how separation is achieved.

The non-polar solution would move faster through the chromatography, as it is more soluble in the solution and interacts less with the silica gel. As a result, the non-polar compound would end up further up the TLC column relative to the polar compound.

2. Explain why the size of spot in TLC matters - what happens if the spot is too big or too small?

Too big of a spot will result in streaks rather than distinct spots for the different compounds, and too small of a spot will result in no clear visible spots.

3. Explain how to prepare a TLC lane of a co-spot with two compounds. Indicate the two possible outcomes and the interpretation of each outcome.

For a lane with a co-spot of two compounds, a drop of each compound will be added to the same spot. If the compounds are the same, then there will be only one spot after chromatography, and if they are different, there will be 2 spots corresponding to each of the different compounds after chromatography.

Result and Analysis - TLC of Hydroxyacetophenone Isomers

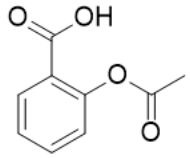

Figure 1. Acetylsalicylic Acid, Rf = 0.64 (TLC conditions: silica gel as stationary, and 75:25 ethyl acetate / petroleum ether as mobile phases)

The silica gel stationary phase is a negatively charged stationary phase, meaning that polar -H bonds would increase solubility with the stationary phase, decreasing the Rf of the compound. Because Acetylsalicylic acid only has one -OH bond (a bond that usually results in the formation of a negatively charged ion and one proton), it experiences minimal interaction with the silica gel. The mobile phase is largely non-polar, which interacts favorably with the aromatic ring and the ketones in acetylsalicylic acid, and good solubility with the mobile phase increases the Rf value.

Result and Analysis - Analysis of an Unknown Analgesic Mixture

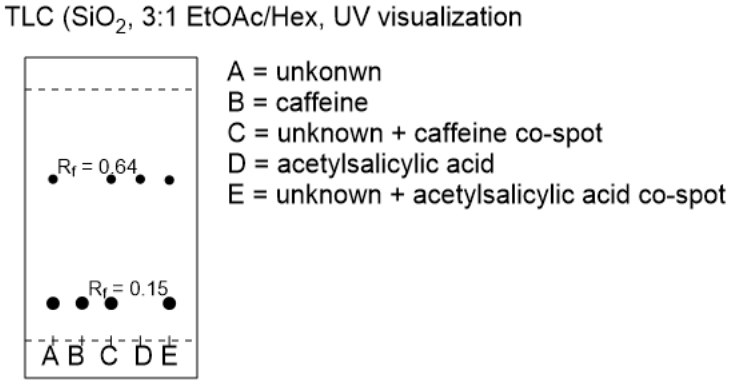

Figure 2. TLC of unknown mixture ID#593 versus known standards.

The 2 compounds were identified as caffeine and acetylsalicylic acid. This was determined by first running 5 lanes: 1 with the unknown, and 4 with each of the standards. Based off of the resulting spot locations, caffeine and acetylsalicylic acid were identified as the two most likely matches. This was then confirmed by running an additional 5-lane TLC with the unknown, caffeine, acetylsalicylic acid, and co-spots with the unknown and each standard (shown in figure 2). The Rf of the 2 unknown spots were 0.15 and 0.64, which matched with caffeine (0.15) and acetylsalicylic acid (0.64). Additionally, the co-spot tests yielded the same 2 spots as the unknown, but with slightly stronger spots corresponding to the standard the co-spots were mixed with.

Conclusion (Parts A and B)

The two compounds of the unknown were identified as caffeine and acetylsalicylic acid. This was based off of the Rf values in the unknown (0.15 and 0.64), which corresponded closest to caffeine and acetylsalicylic acid, respectively. This was confirmed by co-spot analysis, which resulted in the same 2 spots as the unknown, confirming the identify of the unknown compounds.

Part C Column Chromatography

Introduction (Part C)

The objective of this part of the experiment was to separate a 3-component mixture using column chromatography, and then to use TLC to analyze the resulting fractions.

Column chromatography is an effective means of separating different components of a solution, which has a variety of uses, including in forensics and crime scene analysis.

Column Chromatography – Interpretation of Result

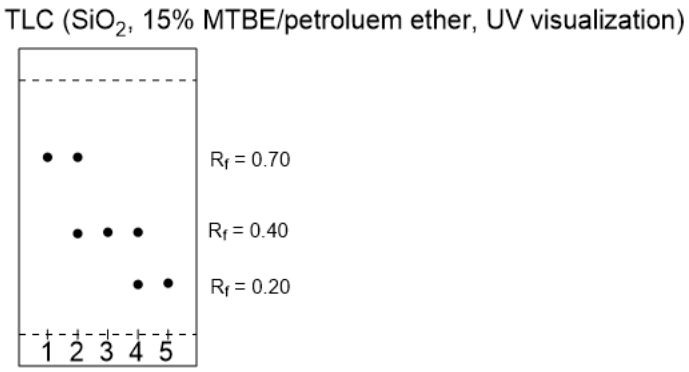

Figure 3. TLC of 5 column chromatography fractions showing **complete** separation.

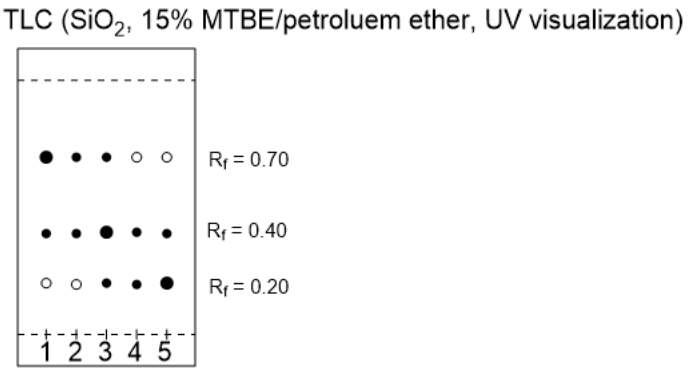

Figure 4. TLC of 5 column chromatography fractions showing **incomplete** separation.

Figure 3 shows complete separation of the components because there are lanes that only contain a single spot, meaning that there is only one compound in that fraction. For instance, lanes 1, 3, and 5 are each a pure sample of a different compound, meaning that all three compounds were able to be isolated.

Figure 4 shows incomplete separation because, although the intensities of the compounds change for each fraction, the fractions still contain multiple compounds, meaning that none of the fractions contain a pure sample of any single compound.

Scientific Principles (Column Chromatography)

1. In column chromatography, a non-polar solvent is typically used, and the column contains silica gel as a stationary phase, which is polar. So, the less polar a compound, the less it will interact with the silica gel, and the faster it will move through the column. Therefore, the less polar compound will elute first.
2. If a compound struggles to make it through the column even after a sufficient volume of mobile phase has been used, pressurizing the column could help to speed up the filtration. This will help if the filtration is simply taking a long time. However, if very little of a compound leaves the column even with a lot of mobile phase being added, it is possible that the compound in question is very polar, and interacts minimally with the non-polar mobile phase, at least compared to its interactions with the silica gel polar stationary phase. In this case, adjusting the mobile phase slightly to make it more polar will increase the compound's solubility with the mobile phase, allowing it to be moved through the column more effectively.