Experiment 1:
Thin Layer Chromatography

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Protocol

As outlined in the lab manual ("Experiment 1: Thin Layer Chromatography", Organic Chemistry Laboratory Manual, Department of Chemistry, University of Ottawa, 2014, Exp. 1, pg. 5-7).

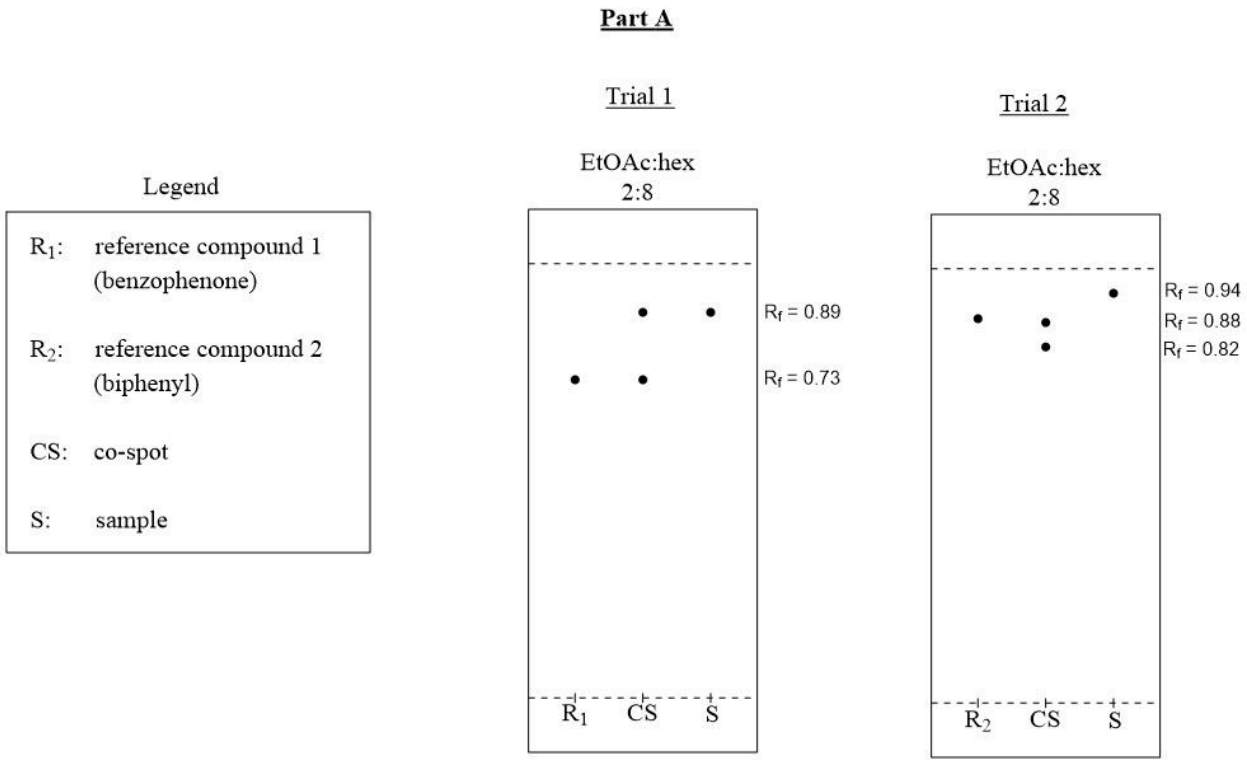
Observations (TLC Analysis)

Unknown Sample ID: 33

- Powdery, white, crystalline solid
- Dissolved quickly in dichloromethane
- Sample will be used in every TLC trial

Note: the following TLC diagrams are based on the template provided in the lab manual ("Experiment 1: Thin Layer Chromatography", Organic Chemistry Laboratory Manual, Department of Chemistry, University of Ottawa, 2014, Exp. 1, pg. 5, Figure: 6)

Figure 1: Two TLC trials that use the same conditions except for the reference compound.



Part A (Figure 1)

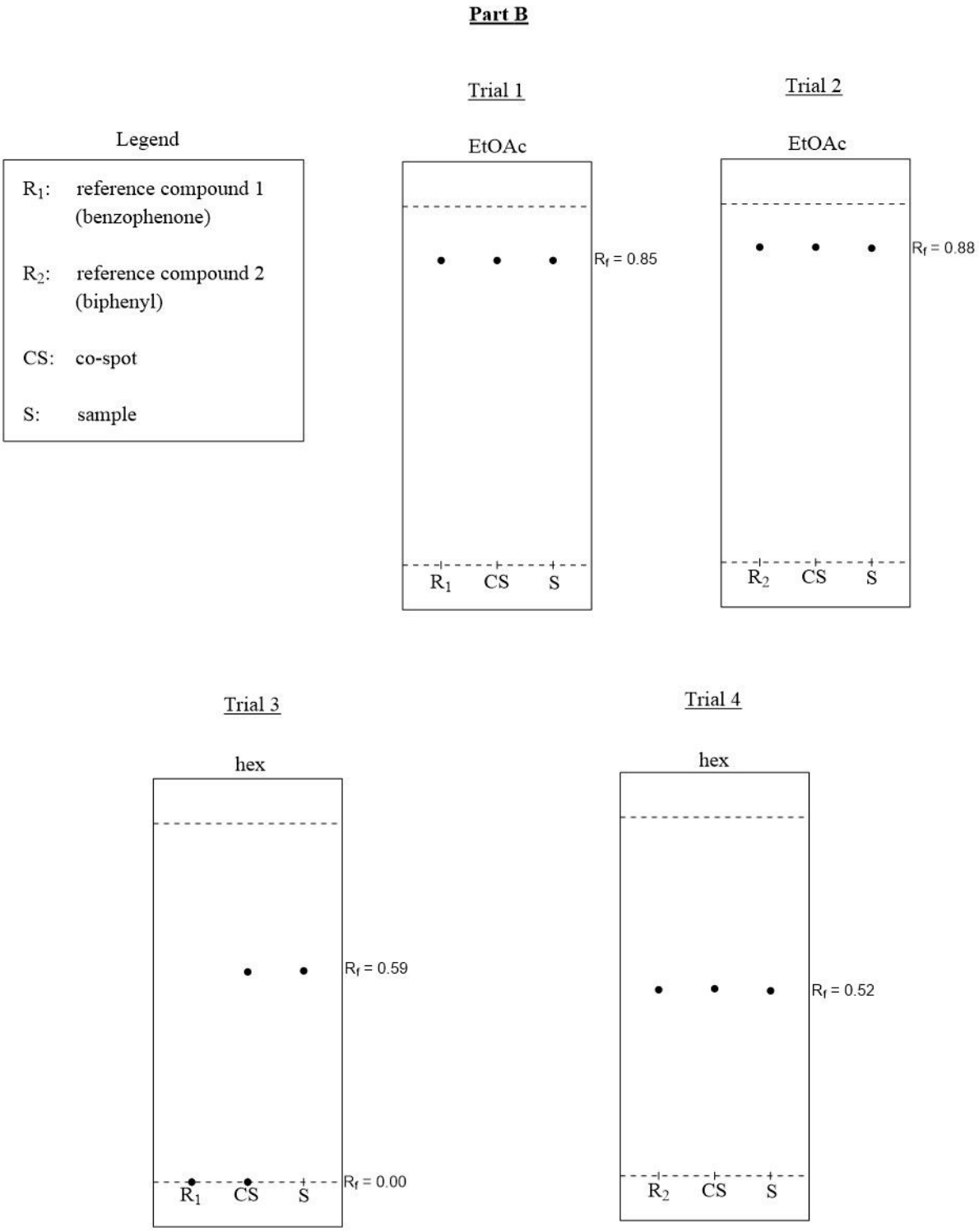
Trial 1

- Compounds in the co-spot lane have significantly separated
- Displacement between reference compound and sample:
    - $\Delta R_f = R_f$ (sample) - R_f (reference)
      $= 0.89 - 0.73$
      $\Delta R_f = 0.16$

Trial 2

- Compounds in the co-spot lane have slightly separated
- Displacement between fast and slow spots in co-spot lane:
    - $\Delta R_f = R_f$ (fast) - R_f (slow)
      $= 0.88 - 0.82$
      $\Delta R_f = 0.04$
- Displacement between reference compound and sample:
    - $\Delta R_f = R_f$ (sample) - R_f (reference)
      $= 0.94 - 0.88$
      $\Delta R_f = 0.06$

Figure 2: Four TLC plate trials. Trial 1 and Trial 2 use ethyl acetate as the mobile phase and use different reference compounds. Trial 3 and 4 use hexanes as the mobile phase and use different reference compounds.



Part B (Figure 2)

Trial 1

- No separation of compounds in co-spot
- Displacement between reference compound and sample:
    - $\Delta R_f = R_f$ (sample) - R_f (reference)
      $= 0.85 - 0.85$
      $\Delta R_f = 0.00$

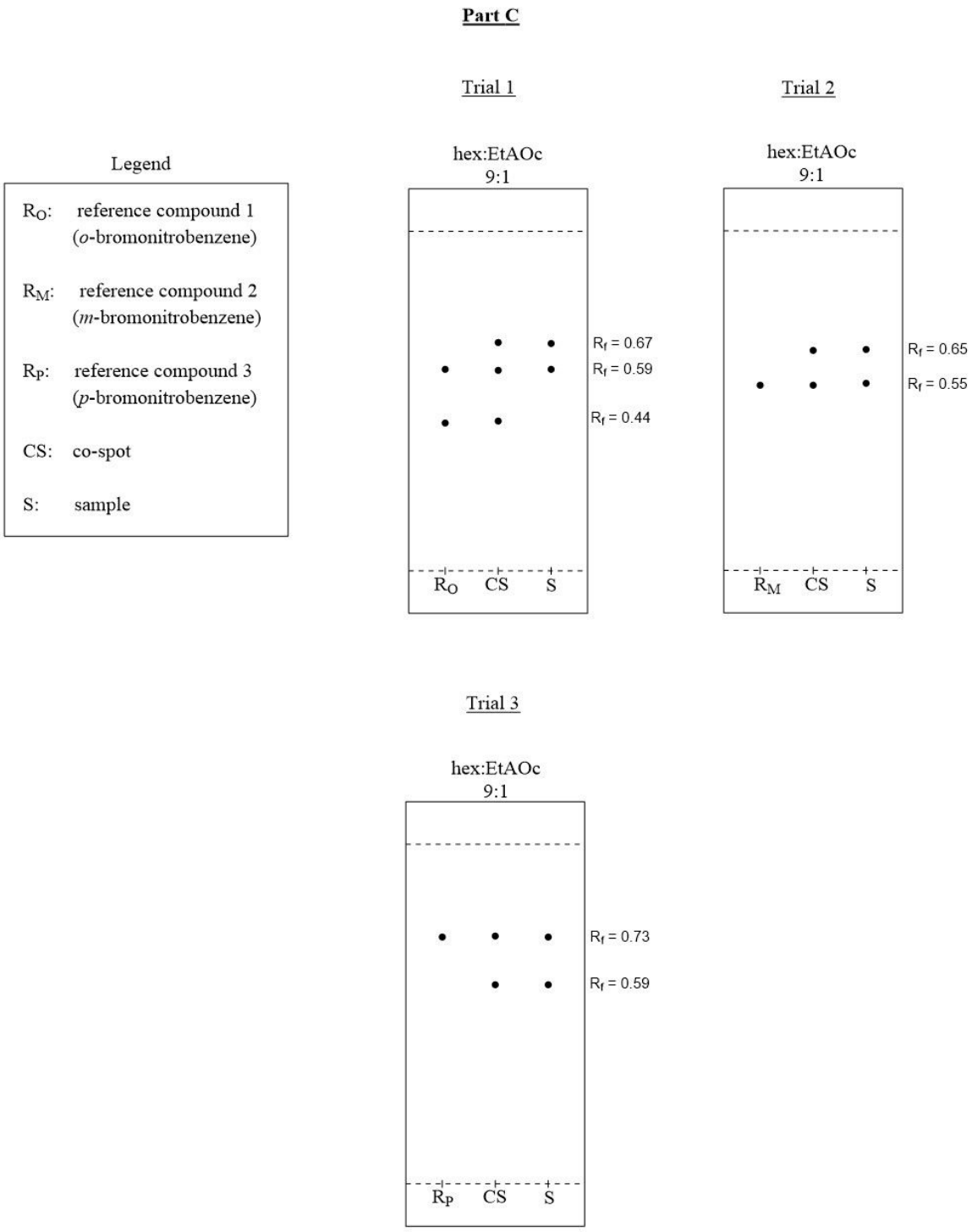
Trial 2

- No separation of compounds in co-spot
- Displacement between reference compound and sample:
    - $\Delta R_f = R_f$ (sample) - R_f (reference)
      $= 0.88 - 0.88$
      $\Delta R_f = 0.00$

Trial 3

- Significant separation of compounds in co-spot lane
- Displacement between reference compound and sample:
    - $\Delta R_f = R_f$ (sample) - R_f (reference)
      $= 0.59 - 0.00$
      $\Delta R_f = 0.59$

Figure 3: Three TLC plate trials, each using the same mobile phase but different reference compounds.



Part C (Figure 3)

Trial 1

- Number of separate spots in reference lane: 2
- Number of separate spots in co-spot lane: 3
- Number of separate spots in sample lane: 2

Trial 2

- Number of separate spots in reference lane: 1
- Number of separate spots in co-spot lane: 2
- Number of separate spots in sample lane: 2

Trial 3

- Number of separate spots in reference lane: 1
- Number of separate spots in co-spot lane: 2
- Number of separate spots in sample lane: 2

Discussion

Part A (Figure 1)

Trial 1:

- Separation of the compounds in co-spot lane indicates a significant difference ($\Delta R_f = 0.16$) in polarity between reference compound and the unknown sample
- Since faster spot in co-spot lane has the same R_f value as unknown sample, faster spot = sample compound (therefore slower spot = reference compound)
- Since sample compound traveled faster, it was less polar than reference compound
    - Mobile phase moves quickly up TLC plate via capillary action
    - Mobile phase has a high ratio of hexanes, which are less polar than the stationary phase (silica gel) [1]
    - Compound that has high R_f value is eluted by the solvent system - can only occur if compound has higher affinity for solvent than for stationary phase
    - Therefore, sample compound must be less polar because it is attracted to the less polar mobile phase
- Slower reference compound was expected
    - Reference compound = benzophenone = ketone (-one suffix)
    - Ketones = polar functional group (has oxygen, which is highly electronegative = more hydrogen bonding = more polar) [2]
    - Polar compounds = higher affinity for the stationary phase, since silica gel is highly polar
    - Therefore polar compounds are slower because of higher affinity for the stationary phase than mobile phase
- Therefore, unknown compound is less polar than ketones

Trial 2:

- Small difference in separation of compounds in co-spot ($\Delta R_f = 0.04$) AND between spots in sample and reference lanes ($\Delta R_f = 0.06$) indicate similar polarities between reference compound and unknown sample
- Both had large R_f values, therefore both traveled fast
- Fast reference & unknown compound was expected
    - Based on previous trial, unknown sample = less polar
    - Reference compound = biphenyl = aromatic compound (-phenyl suffix = benzene)
    - Aromatic compounds = less polar [2]
    - Mobile phase = less polar
    - Therefore, both more attracted to mobile phase and get eluted

Part B (Figure 2)

Trials 1 & 2

- Mobile phase = ethyl acetate = polar solvent [1]
- Stationary phase = silica gel = more polar than the mobile phase
- Same R_f values for all compounds = similar polarity of mobile vs. stationary phase
- Cannot be same compound, different polarities confirmed in previous trials
    - Since mobile phase is polar, has high attraction to stationary phase, therefore travels faster up TLC plate
    - Polar compound (benzophenone) attracted to stationary phase gets eluted because mobile phase also attracted to stationary phase - moves it slightly fast
    - Less polar compound (unknown sample) not polar enough to have higher affinity for silica gel than ethyl acetate, therefore gets eluted by mobile phase - moves slightly fast
    - Ethyl acetate not less polar enough to separate less polar compounds from more polar compounds - not enough difference in polarity between two phases

Trial 3

- Extreme difference in R_f values = extreme difference in polarity
- Mobile phase = hexanes = less polar solvent
- Compound with $R_f = 0.59$ (unknown sample)
    - Traveled up TLC plate = higher affinity for mobile phase = less polar
- Compound with $R_f = 0.00$ (benzophenone)
    - Completely stationary = no affinity for mobile phase = only interacted with stationary phase = highly polar

Trial 4

- No difference in R_f values = same polarity
- As determined in previous trials, both biphenyl and unknown sample are nonpolar, thus have the same affinity for mobile phase, therefore travel same distance

Part C (Figure 3)

Trial 1:

- Separation of compounds in all lanes
    - Since the mobile phase had both hexanes and ethyl acetate, individual compounds may have interacted with either for varying amounts of time, resulting in some reference compounds being eluted by ethyl acetate molecules and some sample compounds being eluted by hexanes
    - Some polar molecules may have had their attraction to the mobile phase disrupted by less polar bonds more often, thus being more attracted to the silica gel, resulting in a smaller R_f value
    - Since unknown compound was dissolved in dichloromethane [1], the compound and dichloromethane separated based on their affinity for each phase
    - Dichloromethane is less polar, therefore it may have been the one that had a higher R_f value in the sample lane

Trial 2:

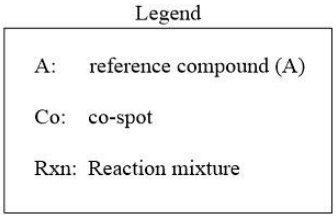
- Separation of compounds only in sample and co-spot lane
    - Separation of compounds in sample lane indicates separation of dichloromethane and unknown compound
    - Since dichloromethane is less polar, it is most likely higher spot
    - Since the reference compound and the unknown compound have the same R_f value, the unknown sample has the same polarity/affinity as *m*-bromonitrobenzene using this specific mobile phase

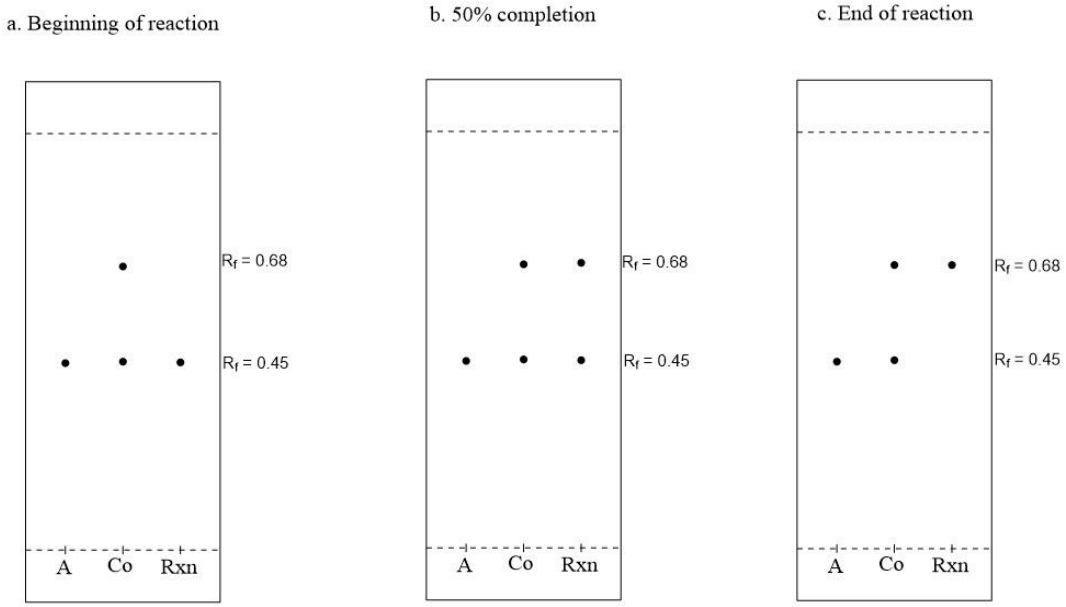
Trial 3:

- Separation of compounds only in co-spot and sample lane (just like Trial 2)
    - The reference compound is higher than the ones in the previous trials
    - This suggests that *p*-bromonitrobenzene is most likely the least polar out of the three reference compounds used in Part C

Questions

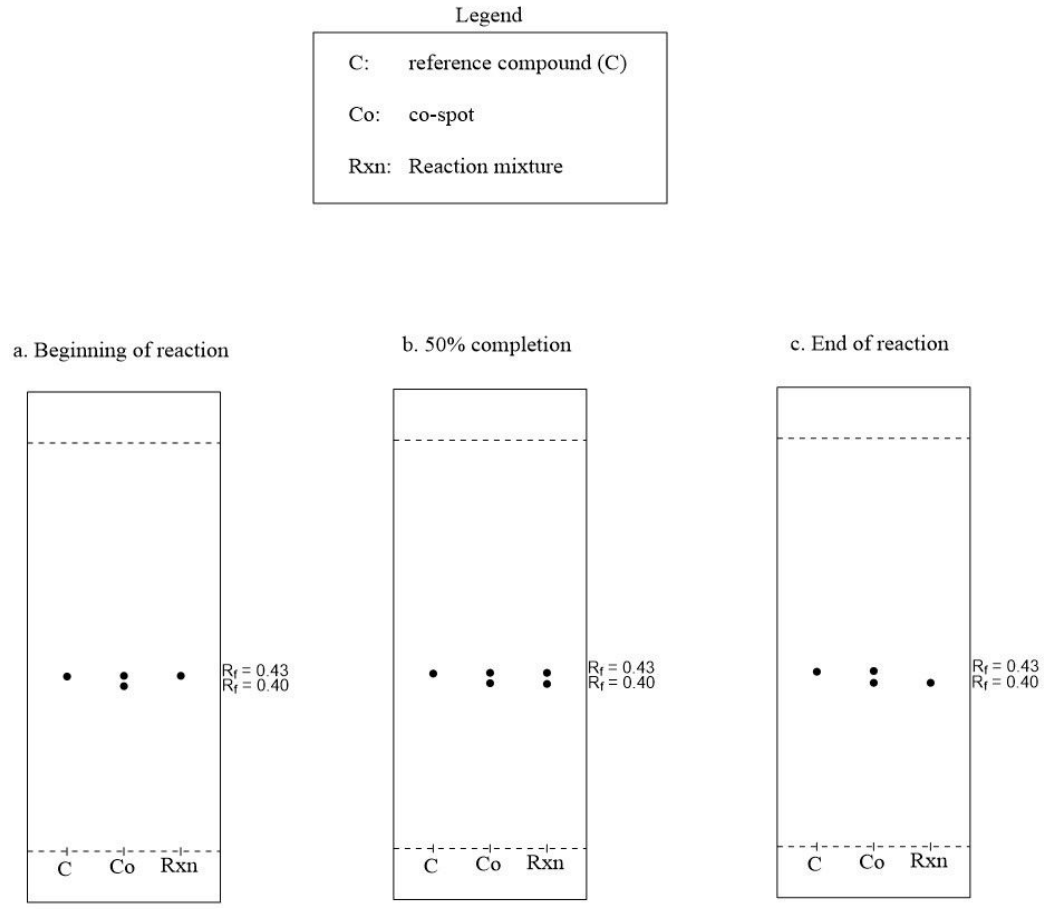
1.
   - Co-spot = mix of sample + reference
   - If co-spot done first, capillary will pick up both compounds, contaminating the other lanes with one of the other compounds that were picked up
   - Doing co-spot last prevents contamination, maintains integrity of lanes
2.
   - Both stationary and mobile phases would have similar polarities
   - Similar polarities would cause similar R_f values between the compounds
   - High difference in polarity between phases allows compounds to interact with the phase corresponding to their polarity, therefore allowing compounds to separate
3.





   - d. Since A is the initial reactant, using A as the reference point allows the student to evaluate whether the reaction progression has begun or not. If the R_f value is the same as that of A, the student can conclude that the reactants have not been converted into products.

4.

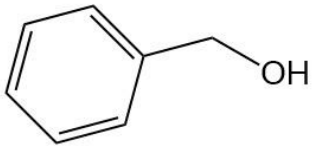


- d. For this particular reaction, the co-spot helps visually differentiate compound C and compound D, since R_f values for each compound are extremely close. Without the co-spot, visually differentiating between which compound is which would be much more difficult.

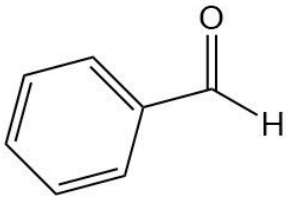
5.

- a.

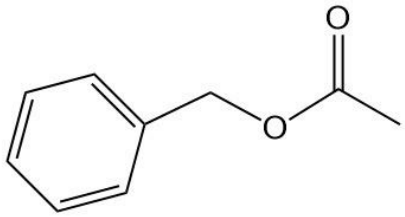
i.



Benzyl alcohol

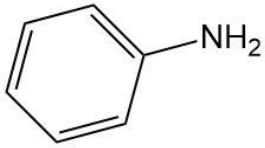


Benzaldehyde

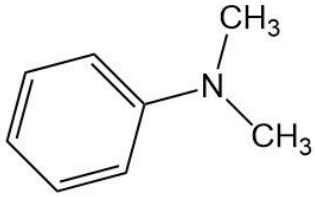


Benzyl acetate

ii.



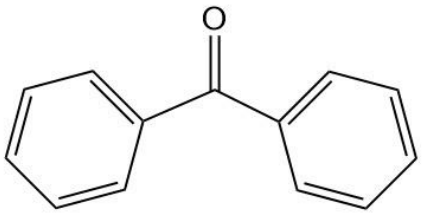
Aniline



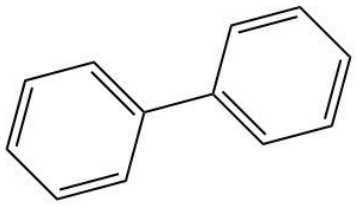
N,N-dimethylaniline

Naphthalene

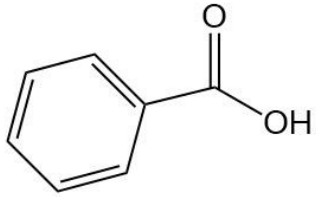
iii.



Benzophenone



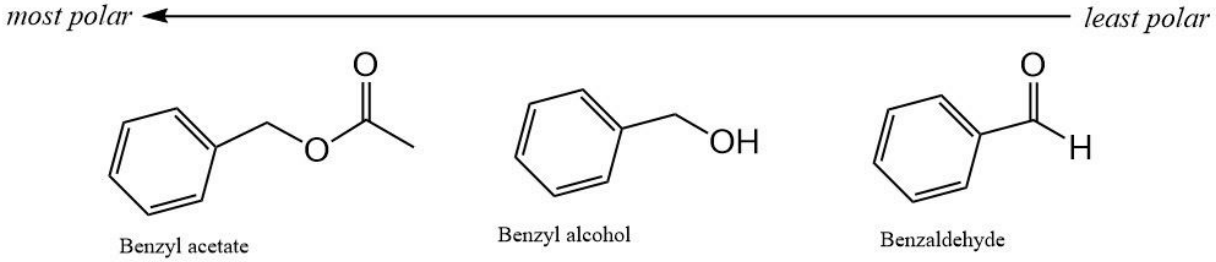
Biphenyl



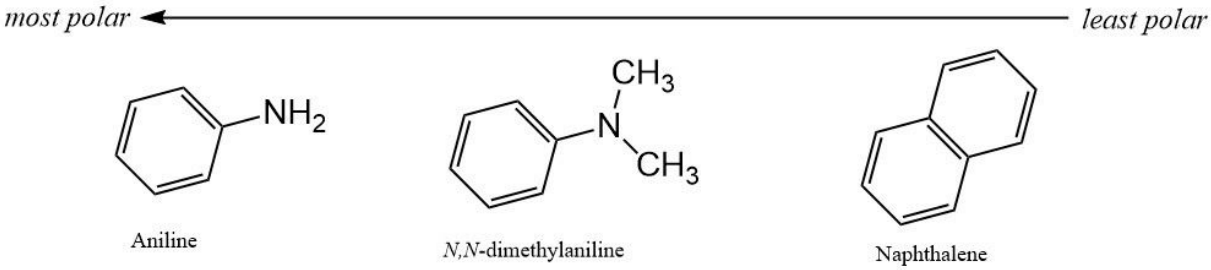
Benzoic acid

- b.

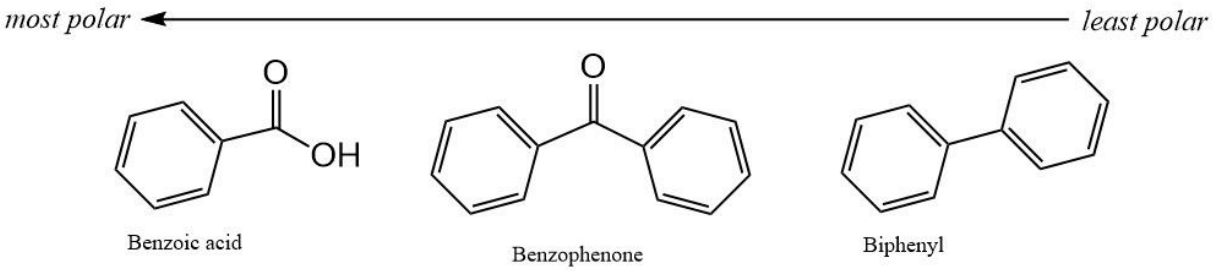
i.



ii.



iii.



- c. The most polar molecules are the ones that are able to participate in hydrogen bonding [3]. This includes molecules which contain electronegative atoms that have available bonding sites (lone pairs) and contain the most dipole moments within the molecule (including electronegative atoms bonded to hydrogen). Benzyl acetate has an ester group, which contains 2 oxygens - highly electronegative atoms that can participate in hydrogen bonding. Aniline contains a primary amine, thus possessing 2 dipole moments, since nitrogen is more electronegative than the 2 hydrogens. Benzoic acid contains a carboxylic acid group, which is the most polar functional group.

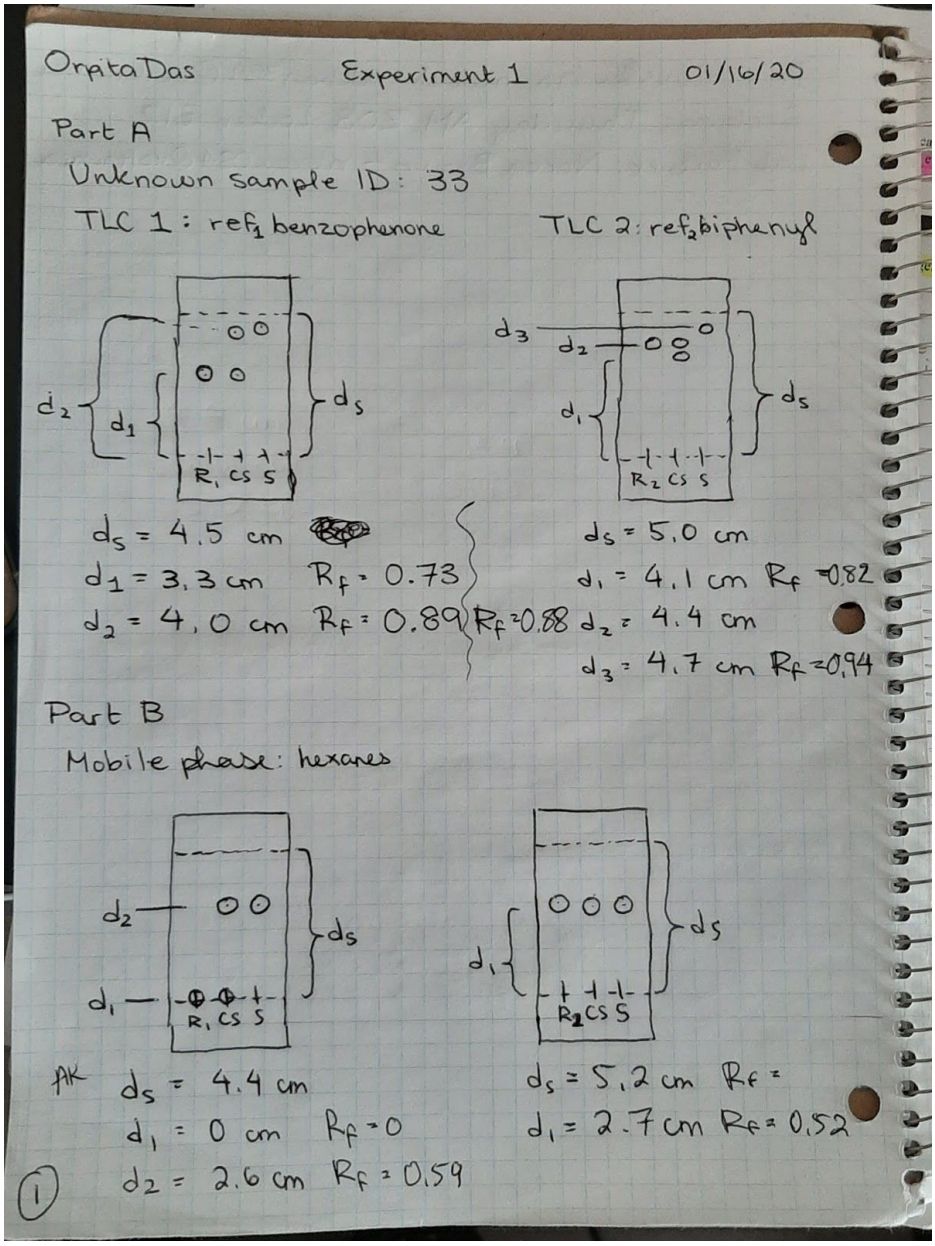
References

[1]: “Experiment 1: Thin Layer Chromatography”, Organic Chemistry Laboratory Manual, Department of Chemistry, University of Ottawa, 2014, Exp. 1, pg. 2, Table 2

[2]: “Experiment 1: Thin Layer Chromatography”, Organic Chemistry Laboratory Manual, Department of Chemistry, University of Ottawa, 2014, Exp. 1, pg. 2, Table 3

[3]: Soczewinski, E., *Analytical Chemistry*, **41**(1) (1969) 179-182.

Raw Data



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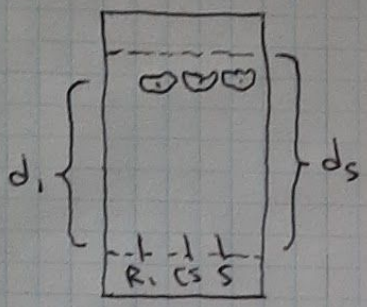
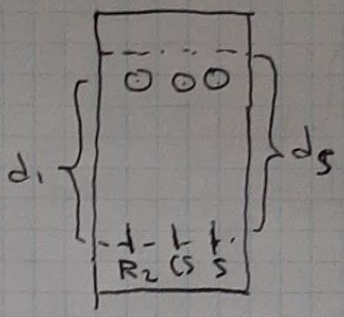
- Mobile phase: ethyl acetate



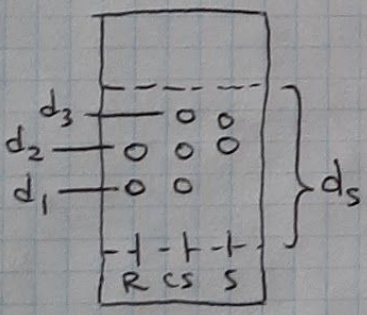
$d_s = 5.5$ cm
$d_1 = 4.7$ cm $R_f = 0.85$



$d_s = 5.0$ cm
$d_1 = 4.4$ cm $R_f = 0.88$

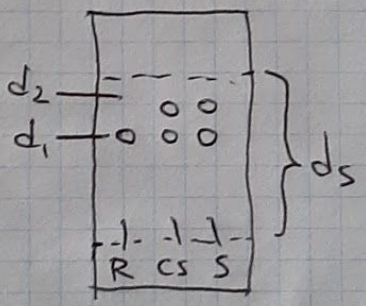
Part C

- Trial O



$d_s = 3.9$ cm
$d_1 = 1.7$ cm $R_f = 0.44$
$d_2 = 2.3$ cm $R_f = 0.59$
$d_3 = 2.6$ cm $R_f = 0.67$

Trial M



$d_s = 4.0$ cm
$d_1 = 2.2$ cm $R_f = 0.55$
$d_2 = 2.6$ cm $R_f = 0.65$ AK

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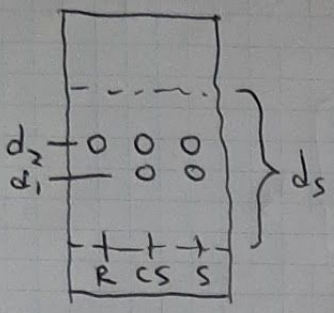
Trial P



$d_s = 4.4$ cm

$d_1 = 2.6$ cm $R_f = 0.59$

$d_2 = 3.2$ cm $R_f = 0.73$

AK