Protocol

Refer to experiment 1 in lab manual (CHM 1321 Introductory Organic Chemistry Laboratory Manual 2018; Dr. Tony Durst, Dr. Tito Scaiano, Dr. William Ogilvie, Dr. Alison Flynn; pp. 13-19)

Modifications:

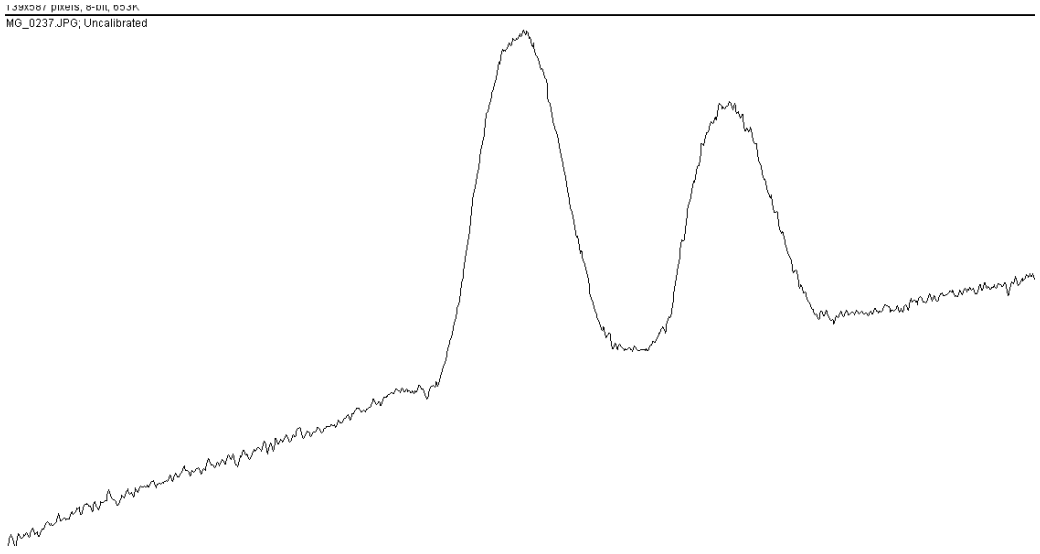
Step in experiment	Modification
Part C, Step 1	2mL of the unknown was not obtained at the burette, but rather first in a beaker and then measured in a graduated cylinder.

Observations:

Substance	Qualitative Observation
Unknown compound #56	White, small coarse powder, took a long time to dissolve completely in dichloromethane
Ethyl acetate	Very strong odour resembling nail polish
Hexanes	Colourless, transparent
Biphenyl	Colourless, transparent
Benzophenone	Colourless, transparent
Unknown ZZ	Yellow colour

Calculations:

Graph 1. Chromatogram of Unknown ZZ demonstrating absorbance by the areas under the peaks, obtained from ImageJ (from left to right, *meta*-bromonitrobenzene and *ortho*-bromonitrobenzene)



Determining R_f values

Solving R_f value for: Part A, reference solution: benzophenone (Table 4)

d_1 = 4.1 cm

d_s = 4.5 cm

$R_f = \frac{d1}{ds}$

$R_f = \frac{4.1\ cm}{4.5\ cm}$

R_f = 0.91

Determining total absorbance

Table 1. Area under the peaks of Graph 1 where 1 is *meta*-bromonitrobenzene and 2 is *ortho*-bromonitrobenzene, obtained from ImageJ

	Area
1	38123.927
2	24176.321

Total area = A1 + A2

Total area = (38, 123.927) + (24, 176.321)

Total area = 62, 300.248

Area = absorbance

∴ total absorbance = 62, 300.248

Calculating percentage of absorbance

% absorbance of meta = $\frac{absorbance\ of\ meta}{total\ absorbance} \times 100\%$

= $\frac{38{,}123.927}{62{,}300.248} \times 100\%$

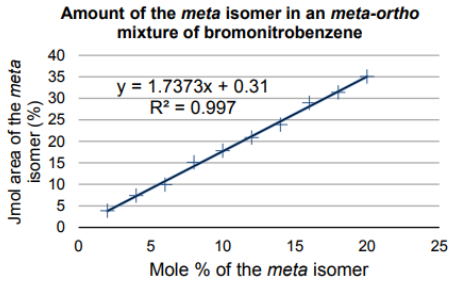
= 61.2 %

% absorbance of ortho = 100% - 61.2%

= 38.8%

Calculating mole percentage

Graph 2. Calibration curve of the "amount of the *meta* isomer in an [sic] meta-ortho mixture of bromonitrobenzene", obtained from Brightspace



y = % absorbance of meta = 61.2%

y = 1.737x + 0.31

where x = mole % of meta

Solving for x:

$y - 0.31 = 1.737x$

$(61.2\%) - 0.31 = 1.737x$

$\frac{60.89\%}{1.737} = x$

$35.05\% = x$

∴ mole percentage of the meta isomer in solution ZZ = 35.05%

Mole % of ortho isomer = 100% - 35.05%

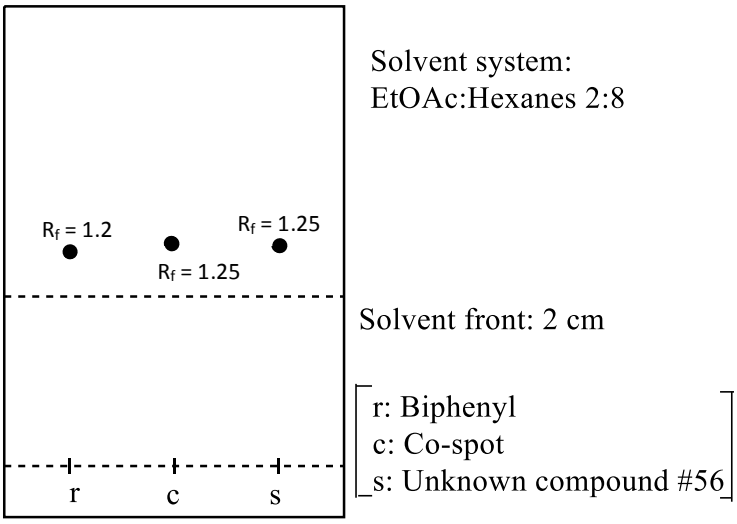
= 64.95%

Table 2. Summary table of calculations performed

Information required	Calculation
Rf value: Part A, reference solution: benzophenone (Table 4)	0.91
% Absorbance of *ortho* isomer	38.8%
% Absorbance of *meta* isomer	61.2%
Mole % of *ortho* isomer	64.95%
Mole % of *meta* isomer	35.05%

TLC Plates

Figure 1. Part A – Solvent system is EtOAc:Hexanes 2:8 and reference solution is biphenyl



Note: The spot in the co-spot lane represents the overlapping of both biphenyl and unknown compound #56

Table 3. Part A – Rf values calculated for when solvent system is EtOAc:Hexanes 2:8 and reference solution is biphenyl

Compound	**Location**	**Rf value**
Biphenyl	Reference lane (r)	1.2
	Co-spot lane	1.25
Unknown compound #56	Co-spot lane	1.25
	Sample lane (s)	1.25

Figure 2. Part A – Solvent system is EtOAc:Hexanes 2:8 and reference solution is benzophenone

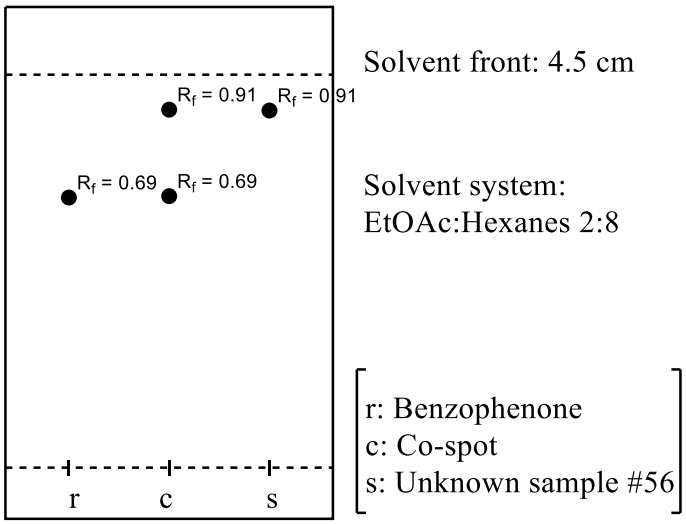
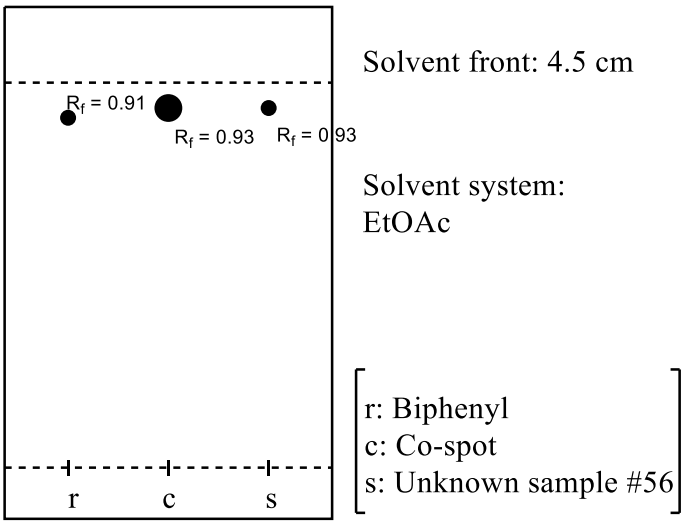


Table 4. Part A – Rf values calculated for when solvent system is EtOAc:Hexanes 2:8 and reference solution is benzophenone

Compound	**Location**	**Rf value**
Benzophenone	Reference lane (r)	0.69
	Co-spot lane (bottom spot)	0.69
Unknown compound #56	Co-spot lane (top spot)	0.91
	Sample lane (s)	0.91

Figure 3. Part B – Solvent system is EtOAc and reference solution is biphenyl

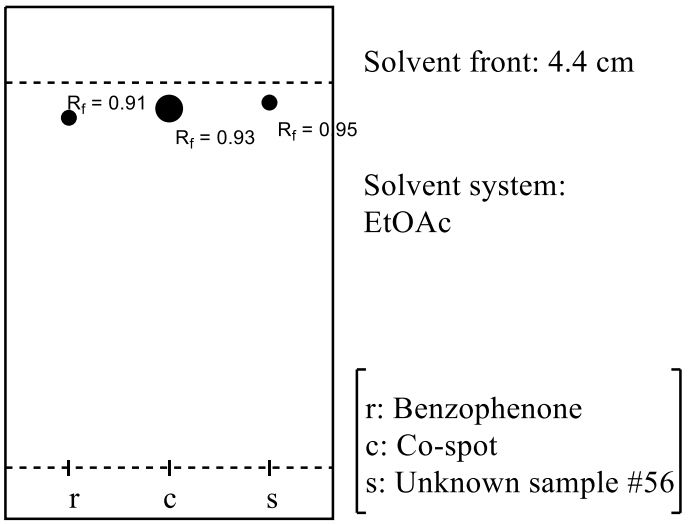


Note: The spot in the co-spot lane represents the overlapping of both biphenyl and unknown compound #56

Table 5. Rf values calculated for when solvent system is EtOAc and reference solution is biphenyl

Compound	**Location**	**Rf value**
Biphenyl	Reference lane (r)	0.91
	Co-spot lane	0.93
Unknown compound #56	Co-spot lane	0.93
	Sample lane (s)	0.93

Figure 4. Part B – Solvent system is EtOAc and reference solution is benzophenone

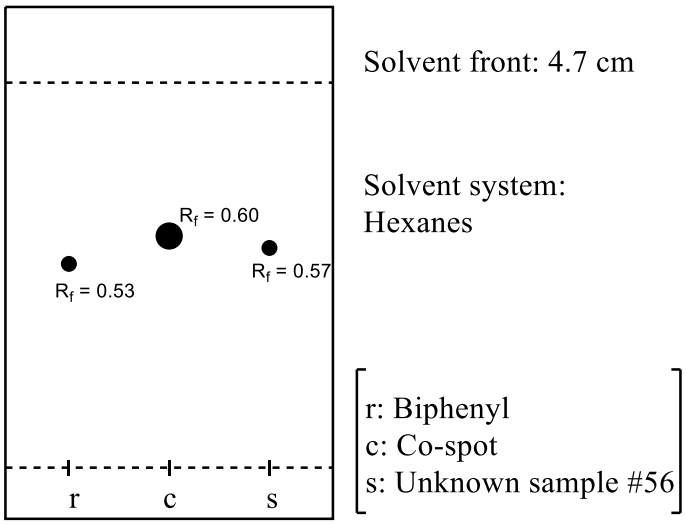


Note: The spot in the co-spot lane represents the overlapping of both benzophenone and unknown compound #56

Table 6. Rf values calculated for when solvent system is EtOAc and reference solution is benzophenone

Compound	**Location**	**Rf value**
Benzophenone	Reference lane (r)	0.91
	Co-spot lane	0.93
Unknown compound #56	Co-spot lane	0.93
	Sample lane (s)	0.95

Figure 5. Part B – Solvent system is hexanes and reference solution is biphenyl



Note: The spot in the co-spot lane represents the overlapping of both biphenyl and unknown compound #56

Table 7. Rf values calculate for when solvent system is hexanes and reference solution is biphenyl

Compound	**Location**	**Rf value**
Biphenyl	Reference lane (r)	0.53
	Co-spot lane	0.60
Unknown compound #56	Co-spot lane	0.60
	Sample lane (s)	0.57

Figure 6. Part B – Solvent system is hexanes and reference solution is benzophenone

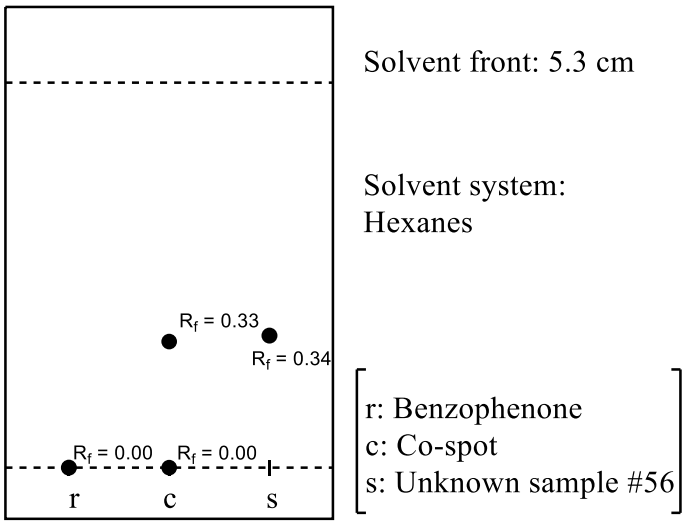


Table 8. Rf values calculated for when solvent system is hexanes and reference solution is benzophenone

Compound	**Location**	**Rf value**
Benzophenone	Reference lane (r)	0.00
	Co-spot lane (bottom spot)	0.93
Unknown compound #56	Co-spot lane (top spot)	0.33
	Sample lane (s)	0.34

Figure 7. Part C – Solvent system is Hexanes:EtOAc 9:1 and reference compound is A (*ortho*-bromonitrobenzene)

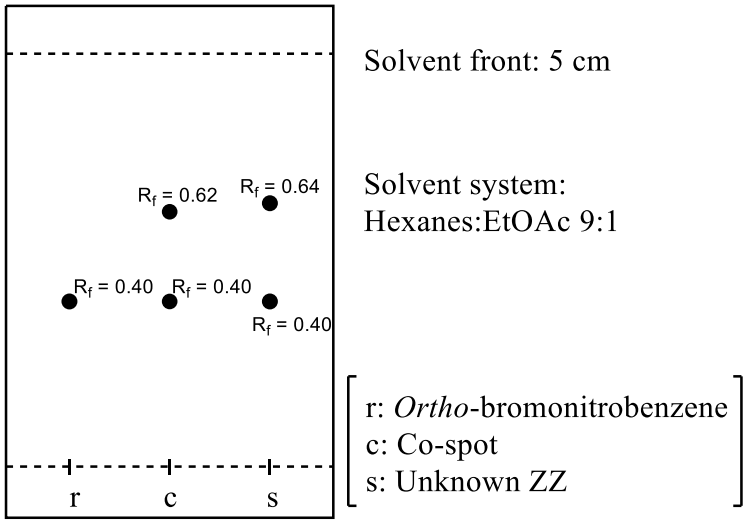


Table 9. Rf values calculated for when solvent system is Hexanes:EtOAc 9:1 and reference compound is A (*ortho*-bromonitrobenzene)

Compound	**Location**	**Rf value**
Ortho-bromonitrobenzene	Reference lane (r)	0.40
	Co-spot lane (bottom spot)	0.40
Unknown ZZ – Compound #1	Co-spot lane (top spot)	0.62
	Sample lane (top spot)	0.64
Unknown ZZ – Compound #2	Co-spot lane (bottom spot)	0.40
	Sample lane (bottom spot)	0.40

Figure 8. Part C – Solvent system is Hexanes:EtOAc 9:1 and reference compound is B (*meta*-bromonitrobenzene)

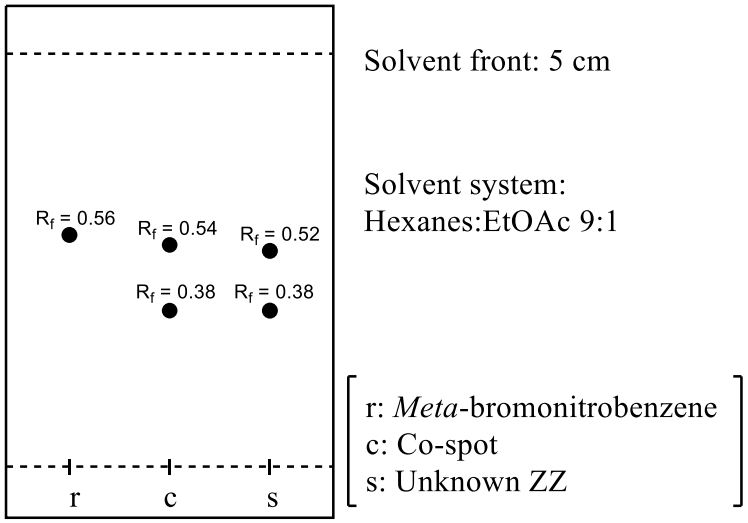


Table 10. Rf values calculated for when solvent system is Hexanes:EtOAc 9:1 and reference compound is B (*meta*-bromonitrobenzene)

Compound	**Location**	**Rf value**
Meta-bromonitrobenzene	Reference lane (r)	0.56
	Co-spot lane (top spot)	0.54
Unknown ZZ – Compound #1	Co-spot lane (top spot)	0.54
	Sample lane (top spot)	0.52
Unknown ZZ – Compound #2	Co-spot lane (bottom spot)	0.38
	Sample lane (bottom spot)	0.38

Figure 9. Part C – Solvent system is Hexanes:EtOAc 9:1 and reference compound is C (*para*-bromonitrobenzene)

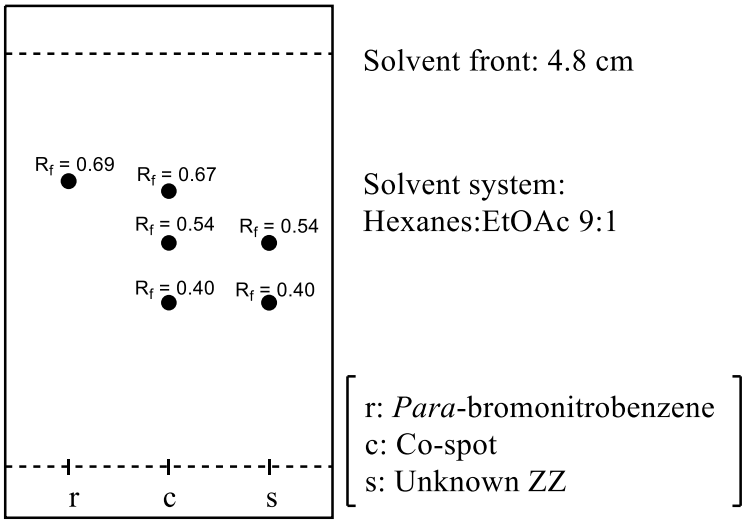


Table 11. Rf values calculated for when solvent system is Hexanes:EtOAc 9:1 and reference compound is C (*para*-bromonitrobenzene)

Compound	**Location**	**Rf value**
Para-bromonitrobenzene	Reference lane (r)	0.69
	Co-spot lane (top spot)	0.67
Unknown ZZ – Compound #1	Co-spot lane (middle spot)	0.54
	Sample lane (top spot)	0.54
Unknown ZZ – Compound #2	Co-spot lane (bottom spot)	0.40
	Sample lane (bottom spot)	0.40

Discussion

Justification of protocol

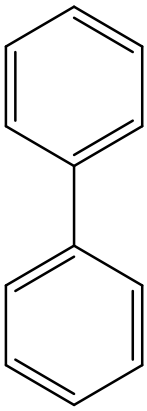
Table 12. Justification of protocol

Important part of protocol	Importance
Place the lid on the developing jar (before & while the plates are in the jar)	This is to minimize the evaporation of the solvent system as organic compounds are highly volatile.
Place TLC plates in developing jar where the pencil line is above the top of the solvent level	This is very important because if the pencil/start line is below the solvent level, then the compounds will dissolve into the solvent and will not show up on the TLC plate (since they will not have eluted).
Mark the solvent finish line when it comes out of the developing jar	This is crucial to calculating the R_f value, as this is d_s. If the solvent finish line is improperly or not at all marked, the R_f value would not be accurate or even determined.
Part A: Preparing TLC plates with biphenyl and benzophenone	Allows for comparison of R_f values to determine what the unknown compound was.
Part A: Using a solvent system of 2:8 EtOAc:Hexanes	This solvent system was essentially neutral, and so R_f values were solely based on the compound's affinity for the silica gel (no interference).
Part B: For each new solvent, preparing TLC plates with biphenyl and benzophenone	Allows for complete analysis when the polarity of the solvent is changed, as well as when the polarity of the reference compound is changed. Allows to see interactions between the two, and the solvent's effects on the R_f values.
Part B: Using pure EtOAc & hexanes as two solvent systems	Allows the opportunity to see the effects of solvent systems' polarities on the compounds' R_f values.
Part C: Use reference compounds *ortho*-, *meta*-, and *para*-bromonitrobenzene	Essential to determining the composition of Unknown ZZ.
Part C: Use ImageJ and calibration curves	Image J helped determine the percentage of adsorbance of the compounds in Unknown ZZ. This was used, with the calibration curves, to then determine the mole percentage of each compound in the unknown.

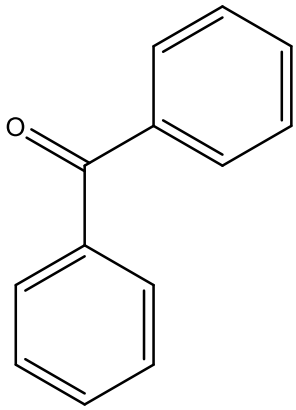
Part A

- Looking at the TLC plate in Figure 2, the sample and the reference solution (benzophenone) do not have the same R_f values. The sample moved much higher and closer to the water front (R_f was 0.91) whereas the benzophenone solution went up a little past the middle (R_f of 0.69).
- Looking at the TLC plate in Figure 1, the sample and the reference solution (biphenyl) have almost the exact same R_f values. This indicates that the unknown compound #56 was biphenyl.

- The R_f values obtained from Figure 2 are: 0.91 for biphenyl and 0.69 for benzophenone.
- The solvent system in this case was EtOAc:Hexanes 2:8, which, since it is relatively neutral, most likely had no effect on the compounds moving along the plate.
- A large R_f means that the compound must have a lower affinity for the silica gel (a polar adsorbent) and thus be non-polar. This makes sense when looking at biphenyl, which had a large R_f of 0.91, as its structure is of a phenyl group attached to a benzene ring (see below) – both of which are aromatic compounds and thus non-polar.



- A smaller Rf means that the compound had a higher affinity for the polar silica gel, where intermolecular forces between the two are slowing down the compound from rising on the plate. This makes sense when looking at benzophenone, which had a smaller Rf of 0.69, as, unlike biphenyl, its structure has a ketone functional group in the middle (see below). This functional group resulted in a polar compound due to uneven distribution of charge.



Part B

- Two different solvent systems of different polarities were used: ethyl acetate and hexanes.
- Ethyl acetate is more polar than hexane because of its ester (which gives two highly. electronegative oxygen atoms). A hexane is a saturated hydrocarbon, which is always non-polar.
- *Ethyl Acetate*
    - The spots moved very close to the solvent front, with all the R_f values being greater than 0.9.

    - This may have happened because the highly polar ethyl acetate interrupted any intermolecular attractions between the sample and/or reference compound and the silica gel.
    - Benzophenone is a polar molecule, as established in Part A, which increases its affinity for the polar silica gel. When this affinity is suddenly redirected to a more polar compound (in this case EtOAc), the benzophenone follows the eluent higher up on the plate.
    - Even though all the spots moved up, similar to the results of Part A, biphenyl (a non-polar compound) had the highest R_f value. This was able to be seen on the plate where the reference compound was benzophenone and the unknown was biphenyl.
- *Hexanes*
    - The spots moved down, closer to the starting line. This means that the Rf values decreased, especially compared to the results with ethyl acetate as the solvent.
    - It has been previously seen that biphenyl has the highest Rf value, regardless of solvent, and with the hexanes solvent this still holds true.
    - Figure 5 shows how the reference and unknown (both of which are biphenyl), had approximately the same Rf values: between 0.53 and 0.60.
        - This was unexpected because biphenyl is a non-polar compound, so it should dissolve readily in the non-polar hexanes solvent and elute very high[1]. This should mean that it should have a high R_f value, but it does not.
        - This was most likely due to a source of error which will be discussed later.
    - Figure 6 shows that, when the reference was benzophenone, benzophenone didn't move up at all from the starting line, thus it has a R_f value of 0.0.
        - This makes sense as benzophenone is a polar molecule, so it would have intermolecular attractions holding it to the polar silica gel.
        - With a non-polar solvent, there would be no interruptions in its adsorption to the silica gel – resulting in complete immobility.
    - The unknown compound (biphenyl) had Rf values of 0.33 (co-spot lane) and 0.34 (sample lane)

Part C

- Figure 7 shows that the R_f value for the reference compound (*o*-bromonitrobenzene) was 0.40 and compound #2 in the sample lane also had an R_f value of 0.40. This indicates *o*-bromonitrobenzene is a compound in Unknown ZZ.
- Figure 8 shows that the R_f value for the reference compound (*m*-bromonitrobenzene) was 0.56 and compound #1 in the sample lane had an Rf value of 0.52. In the co-spot lane, the highest spot had an Rf value of 0.54.
    - These values should theoretically be the same if it were to be the same compound, however, it is most likely due to errors (that will be discussed) that this didn't happen.

[1] Libretexts. "Thin Layer Chromatography." *Chemistry LibreTexts*, Libretexts, 3 Nov. 2017, chem.libretexts.org/Demonstrations_and_Experiments/Basic_Lab_Techniques/Thin_Layer_Chromatography.

- Since the values are within 0.04 of each other, it can be inferred that *m*-bromonitrobenzene is a compound in Unknown ZZ.
- Figure 9 shows that the R_f value for the reference compound (*p*-bromonitrobenzene) is 0.69, which is completely different from the R_f values in the sample lane: 0.54 for compound #1 and 0.40 for compound #2.
- This indicates that *p*-bromonitrobenzene is not part of Unknown ZZ, and, given its different R_f value, further proves (despite the small differences) that *m*-bromonitrobenzene is a component of Unknown ZZ.
- The mole percent of *o*-bromonitrobenzene in Unknown ZZ was calculated to be 64.95%, and *m*-bromonitrobenzene 35.05%. This is a ratio of 1.8:1 (or 9:5 in integers) *ortho*:*meta*.

Sources of Error

- Not drawing the solvent front line immediately after removing the plate from the developing jar – this is what most likely led to the incorrect R_f values in Figure 1.
- Putting the plate in the developing jar was difficult because, after dropping it in, it had to lean against the side. However, twice, the plates fell the into the solvent and had to be redone. Sometimes after that they didn't fall completely in, but they fell enough that the solvent in the jar may have come above the starting line and dissolved a bit of the compounds.
- There was a very faded spot underneath the reference spot in Figure 9 (*p*-bromonitrobenzene) which seemed to align with the *m*-bromonitrobenzene height. This may be because a previous capillary tube was used to spot the reference compound.
- Some plates were left for longer than others and so the solvent line came very close to the end of the plate
- Some spots were larger as they were smudged at the bottom. This made it difficult to measure the displacement of the compound as it was measured from the centre of the spot and may have resulted in lower d_1s (and thus lower R_f values) than were supposed to be.
- There was what looked like smudge streaks under the spots for biphenyl in Part B of the lab (solvent: hexanes). They seem to be very large spots, which implies a high concentration of spotted biphenyl which may have been too much for the solvent to elute. This in turn resulted in biphenyl, non-polar, not eluting as high up as it theoretically should in a non-polar solvent, hexanes[2]. Thus, the R_f values are less than they should be.

Methods of improvement

- Being ready to immediately draw the solvent line (because it evaporates so quickly). This means having a designated area where the plates will be removed and where pencils are ready to be used only for drawing the solvent line & deciding who will mark the line. This marking of the lines needs to be as efficient as possible to not waste time and lose the solvent line.
- Using a tool (i.e. forceps or clamps) that would allow for ease and full control over where the plates are being placed.

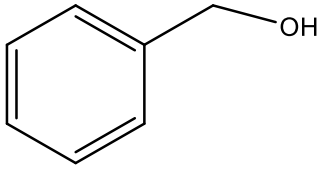
[2] "Principles of chromatography (Article)." *Khan Academy*, www.khanacademy.org/test-prep/mcat/chemical-processes/separations-purifications/a/principles-of-chromatography.

- Have a designated capillary tube throw bin for every 2 stations. Otherwise they're scattered on paper towels or on a work surface and it can be difficult to determine if one has been used or not.
- Prepare all the TLC plates ahead of time so that when the plates are developing, full attention can be given to them to avoid too high solvent front lines.
- Measure height of smudged spots to determine if it's better to measure from the top (if the spot seems absurdly large), or if it's still a reasonable size. Also make sure that a very small amount of liquid is spotted, because larger spots give worse results.
- Instead of immediately starting to spot compounds on to TLC plates, spotting should be practiced beforehand to ensure only small amounts of the compound are being spotted (and not too large amounts like seen in Part B, solvent system: hexanes). Perhaps an even better solution would be an automatic capillary tube, where compounds go up via capillary action, and the amount released would be controlled.

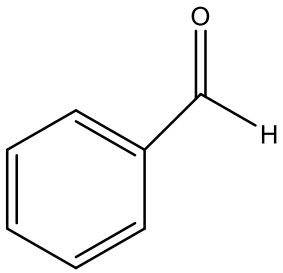
Questions:

1. – Any polar compounds spotted onto a plate will have a great affinity for the (polar) silica gel, so the two will have intermolecular interactions. This attraction (adsorption) slows down any polar compounds going up the plate, thus giving it a lower R_f value.
– Increasing the polarity of the solvent system would interrupt these intermolecular interactions. The more polar the solvent, the more strongly it will interrupt the interactions and pull the polar compound away from the silica gel.
– This will make the compound dissolve into the solvent and thus elute much higher than it did when it was interacting with the silica gel, thus increasing the Rf values of the polar compounds.
– The non-polar compounds would also elute much higher than they already do, thus increasing their R_f value, as well.
– The TLC plate would show that the spotted compounds have all risen very close to the solvent front line and are around the same height. In fact, in the co-spot lane, the two spots may barely be distinguishable.
– Looking at the TLC plates produced in Part B of this lab, except for Figure 7, they all had overlapping spots in the co-spot lane. This shows how having too polar or non-polar eluents can be ineffective when trying to conduct a TLC analysis because there is no separation of compounds.

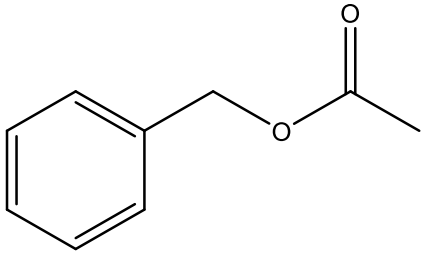
2. a) Benzyl alcohol would have the lowest R_f because it contains a hydroxyl group. This hydroxyl group gives it the highly electronegative oxygen atom, capable of dipole-dipole interactions, and a hydrogen bound to it, capable of hydrogen bonding.



Benzyl Alcohol

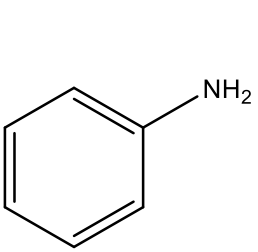


Benzaldehyde

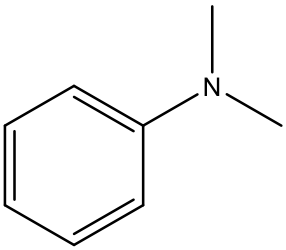


Benzyl acetate

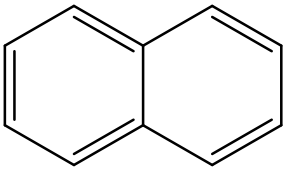
b) Aniline would have the lowest R_f because N is a highly electronegative atom, making it capable of dipole-dipole interactions, and it has hydrogen bound to it, capable of hydrogen bonding.



Aniline

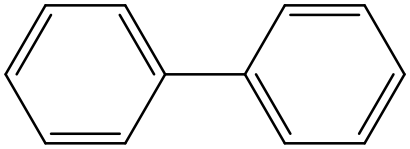


N,N-dimethylaniline

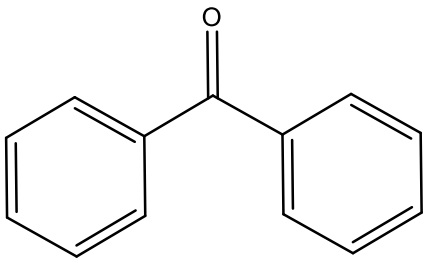


Naphthalene

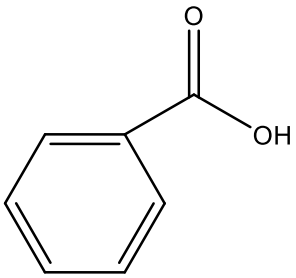
c) Benzoic acid would have the lowest R_f because it has a carboxyl group. The carboxyl group has two oxygen atoms, both causing dipole-dipole interactions, and a hydrogen bound to an oxygen, capable of hydrogen bonding.



Biphenyl



Benzophenone



Benzoic Acid

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