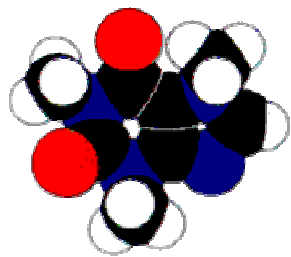


Crazy Caffeine, How Much is In Our Drinks?



Caffeine ($C_8H_{10}O_2N_4$)

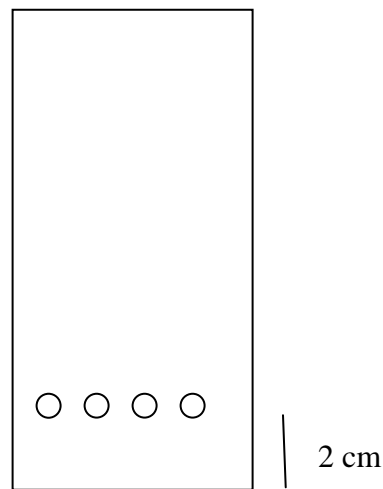
In this exercise the amount of caffeine in various beverages will be determined using Thin Layer Chromatography. The thin layer sheets are composed of silica gel with a florescent coating that has been poured onto an aluminum plate. Known concentrations of caffeine will be run along with the beverage sample of your choice. By comparing known to unknown an accurate estimation can be inferred.

Materials:

Aluminum backed TLC plate
Hot Plate
Pencil
Small Jar
Hand Held UV light
Micropipette (2-20microliter or .002-.02 ml)
Solvent (10% methanol in Ethyl Acetate)
Caffeine Standards
Unknowns

Procedure:

1. Put on your safety glasses.
2. Check that all of the necessary materials are at your lab station.
3. Evenly space at least four pencil dots across the bottom of the TLC plate as shown.
4. In your lab book or on this paper, label the dots as you place your known caffeine solutions in lanes one and two.
5. Place the plate on the hot plate that has been turned on to it's lowest setting.
6. Using the micropipette, deliver 5 ul (.005 ml) to the small dot you have placed. **MAKE SURE** you do this a little at a time. **DON'T** add it all at once. As you add a little wait until it evaporates. Then go ahead and add a little more. The point is to make a very small dot and to make all your spots about the same size.
7. Repeat step 6 for the next known and for all your unknowns.
8. After you have allowed it to cool completely, place the TLC plate very slowly into your small jar (Being careful not to let the spots go under the solvent). This

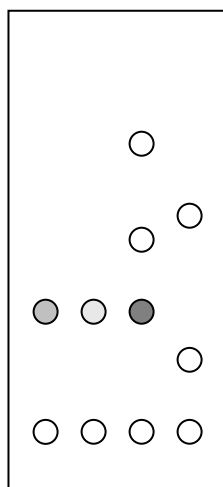


has a flammable solution in it so be very careful and check to ensure that there are no open flames or hot objects nearby.

9. Place the lid back on and watch it until it has nearly reached the top.
10. Take it out and with pencil, mark the furthest point that the solvent traveled.
11. Set it on the table and wait until the plate has dried.
12. Go to the observation area and look at your plate under 254 nm Ultra Violet light. Make a sketch around each dark shape you see in VERY LIGHT pencil.
13. Compare the relative darkness of your two known lanes to each of your unknown lanes.

Results, Calculations, and Conclusions:

Write down all your observations as you compare the darkness of each control spot to your unknowns.



Reading the bottom from left to right.

- Lane one has one spot and it is only Caffeine
- Lane two has one spot and it is in the same location as lane one. It is also only caffeine. Its concentration is half as much as lane one.
- Lane three is tea. It has a spot at the same distance from the original spot as lane one and two. It has a spot that is about twice as dark as lane one.
- Lane four has spots, but none that line up with lane one and two.

Since you know the concentration in lane one (5 mg/ml) and lane three is twice as dark it should have twice as much caffeine or 10 mg/ml.

If you wanted to calculate the amount of caffeine in a glass of tea and the average glass has 400 ml of tea in it.

The calculations are:

$$400 \text{ ml of tea} \times \frac{10 \text{ mg of caffeine}}{1 \text{ ml}} = 4000 \text{ mg of caffeine in your glass of tea}$$