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AP LAB 03I: Thin Layer Chromatography

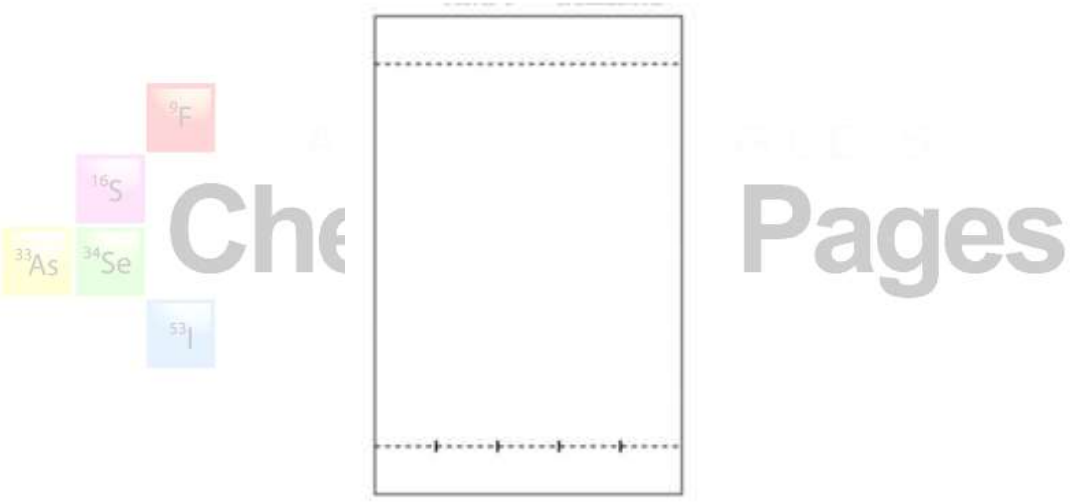
Aim To identify an unknown analgesic via Thin Layer Chromatography

Apparatus 400 mL beaker, capillary tubes, Parafilm, UV light, ruler

Chemicals Developing solvent, TLC plate, five known analgesic samples (caffeine, ibuprofen, naproxen, aspirin, acetaminophen), one unknown analgesic sample

Method

1. Draw a line in PENCIL, approx. 1 cm from the bottom of the TLC plate. Draw lightly on the flaky side of the plate; do not scrape off the coating. Place six tick marks on the line, approx. 1 cm from each other.



2. Immerse the open end of a capillary tube into one of the known samples until some of the solution is drawn into the tube. Very gently press the capillary tube onto the plate at one of the tick marks. Keep the spots small and concentrated by applying the sample at least three times, and allowing the spot to dry between applications.
3. Repeat the procedure in step #2 for each of the five known solutions, and be sure to keep a record of which spot represents each solution.
4. Repeat step #2 for ONE of the unknown samples on to the sixth tick mark, and make a note of which unknown sample you have chosen.
5. Prepare a developing chamber by placing enough solvent (labeled "Developing Solvent") to make a shallow layer on the bottom of a 400 mL beaker. Ensure that there is not so much solvent in the

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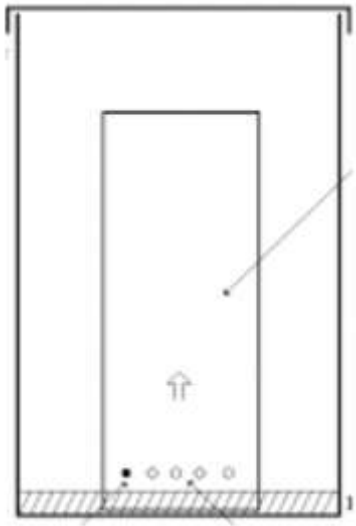
developing chamber that the solvent is deeper than the pencil line where you have spotted your known and unknown samples.



6. Cover the beaker with Parafilm, and allow the solvent to rise to within 1 cm of the top of the plate – this should take 10-15 minutes.
7. Remove the plate, mark the solvent front in PENCIL, and allow it to dry.
8. Visualize the spots by illumination under a UV lamp.
9. Trace around each spot with a pencil, and then measure the distance traveled by each sample. Copy (sketch) your developed plate into the results section.

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Results

Sketch your chromatogram here.

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Conclusion/Calculation

1. Calculate the Rf value for each of the known samples, and that of the unknown sample, and then identify the unknown sample.

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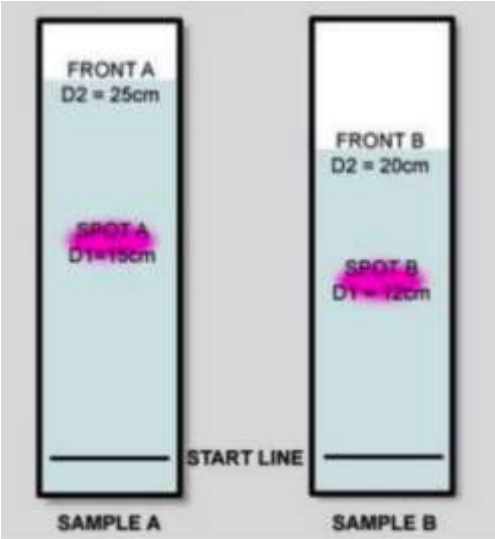
2. The results of a simple chromatography experiment shown below.



D1 = 15 cm for Spot A in Sample A

D1 = 12 cm for Spot B in Sample B

(a) What does the data suggest about the relative affinity of the component of the mixture for the moving phase on the left, when compared to the relative affinity of the component for the moving phase on the right?

(A) The component of the mixture has a greater affinity for the moving phase on the left
(B) The component of the mixture has a greater affinity for the moving phase on the right
(C) The component of the mixture has an equal affinity for the moving phase on both the left and the right
(D) There is no way to tell from the data given

(b) Which of the following will lead to the correct calculation of the R_f value for the component?

(A) 20/12
(B) 12/20
(C) 25/15
(D) 12/15