# Column Chromatography

EXPERIMENT

## Background

We will separate the dyes in Kool-aid using column chromatography. The C-18 column you will be using is a small, reusable cartridge, which is used in industry and research to separate mixtures into their component parts.

## Materials

• One C-18 column

• 2mL of Kool-aid

• One Syringe

• Five sample bottles

• Four Alcohol sample bottles

• One Distilled water bottle

## Procedure

1. Obtain the C-18 column and six glass bottles from your teacher.
2. Obtain a syringe with a rubber stopper from your teacher.
3. Fill the syringe full of 70% alcohol.
4. Attach the syringe to the LONG END of the C-18 column.
5. Holding over a WASTE beaker, push the plunger in SLOWLY. The alcohol will drip out into the waste beaker. If liquid remains on top of the column, remove the syringe and pull the plunger out farther. Reattach syringe to the LONG END of the column to push the remaining liquid through.
6. Repeat steps 3-5 using a pipet full of distilled water.
7. Add 1mL of your Kool-aid sample and let it sink down into the column. Use the plunger to push it along SLOWLY like you did in Step 5. Some may drip into the waste beaker. That is OK. Discard waste collected in the waste beaker.
8. Add a pipet full of distilled water to the column. SLOWLY push the water through the column with the syringe into the bottle labeled SAMPLE #1.
9. Add a pipet full of 8.25% alcohol to the column. SLOWLY push the alcohol through the column with the syringe into the bottle labeled SAMPLE #2.
10. Add a pipet full of 17.5% alcohol to the column. SLOWLY push the alcohol through the column with the syringe into the bottle labeled SAMPLE #3.
11. Add a pipet full of 35% alcohol to the column. SLOWLY push the alcohol through the column with the syringe into the bottle labeled SAMPLE #4
12. Add a pipet full of 70% alcohol to the column. SLOWLY push the alcohol through the column with the syringe into bottle labeled SAMPLE #5.
13. Your teacher has the results of the crime scene sample. Compare your results with the crime scene sample. Do you match? Why or why not?

LIPSTICK: Column Chromatography

TEACHER GUIDE

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| **Materials Needed**  |
| * One Sep-Pak@ C-18 cartridge (column) for each student group
* Kool-Aid@ - different kinds and additives for each suspect
* water
* rubbing alcohol (isopropyl alcohol, 70% by volume, with no color or added scent)
* the following volumetric measures:

25 mL graduated cylinder (for preparation)10 mL graduated cylinder for each student group* 6 small vials or test tubes with labels for each student group
* container to collect liquid waste
* one 10 to 50 mL syringe with male Luer@ tip for each student group
* six 50 to l00 mL plastic bottles for alcohol solutions and water
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**Kool-aid Preparation:**

Prepare suspects Kool-aid samples in the following way:

1. Full packet of grape kool-aid with 300 mL of water
2. Full packet of black cherry kool-aid with 200 mL water
3. Full packet of orange kool-aid with 150 mL water + 50 mL of Solution #1
4. Full packet of berry blue kool-aid with 150 mL water + 50 mL of Solution #1

Alcohol Solution Preparation:

Prepare the following alcohol solutions using rubbing alcohol you buy at the drug store and distilled water.

1. Prepare 40 mL of a 35% isopropyl alcohol solution (20 mL of rubbing alcohol and 20 mL of water). Put the solution into a bottle and label it "35% isopropyl alcohol."

2. Prepare 40 mL of a 17.5% isopropyl alcohol solution (10 mL of rubbing alcohol and 30 mL of water). Put the solution into a bottle and label it "17.5% isopropyl alcohol."

3. Prepare 40 mL of an 8.25% isopropyl alcohol solution (5 mL of rubbing alcohol and 35 mL of water). Put the solution into a bottle and label it "8.25% isopropyl alcohol."

4. Fill one of the remaining solvents or squeeze bottles with water and label it.

5. Fill a fifth bottle with undiluted rubbing alcohol and label it "70% isopropyl alcohol."