**Introduction**

Column chromatography is frequently used in organic chemistry. It is most often used to isolate a chemical so it can be studied and identified, or to separate impurities from a chemical. This extraction is often the first step toward identifying a chemical, determining its structure, and possibly synthesizing it in the lab.

One such compound that was isolated using column chromatography is *vanillin*, the principal flavor component of natural vanilla. Prior to the 1950s, vanilla extract was made by soaking vanilla “beans” (actually the seed pods of a South American orchid) in alcohol for weeks at a time. The process was time-consuming and expensive, and the resulting vanilla extract was very expensive. In the early 1950s, a chemist used column chromatography to isolate vanillin from vanilla beans; over the next few years, he and other chemists studied vanillin’s structure. In 1956, the U.S. Patent Office awarded a patent to two chemists who found a way to synthesize vanillin from the by-products of the wood-pulp industry. Inexpensive imitation vanilla flavoring soon made its way onto grocery store shelves, and the rest is culinary history.

In our lab, we’re going to use column chromatography to separate dyes from a water medium. Our column will use very fine powder – alumina, silica, or a material called “C – 18” – as the packing material. A porous plate known as a *frit* is placed above and below the column to keep it in place. When a mixture is passed through the column, some components are more strongly attracted to the packing material than others. These components “stick” to the column and are retained, while other components pass through in the *effluent*. By selecting proper solvents, we can remove the materials that are sticking to the column and isolate them for further study.

**Objectives**

By doing this lab, students will be able to:

- describe how column chromatography works
- separate a mixture of dyes using a solid-phase extraction tube

**Materials and Equipment**

| Prep-Sep® solid-phase extraction tube | 24-well well plate |
| 5-mL syringe | water |
| Luer-Lok® three-way stopcock/connector | isopropyl alcohol (5%, 10%, 20%, 100%) |
| 100-mL beaker | Kool-Aid® solutions (lemon-lime and grape) |
| Spoon or stirring rod | |

**Safety Considerations**

- As always, you should wear safety glasses/goggles when working in the lab area. If aprons are available, wear one.
- 100% isopropyl alcohol is flammable – keep it away from any sources of sparks or open flame.
- These Kool-Aid solutions will stain your clothes, so be careful when working with them.
- *Do not* drink any of the Kool-Aid solutions. They are overly concentrated, do not contain sugar, and would taste nasty. Besides, do you know where that beaker’s been?
- *Do not* push on the syringe or let the plunger snap out of your fingers when you’re doing your extraction. This will force the liquid back up through the column, dislodging the frits and the packing material; this renders the column unusable. This is known as “blowing” a column and could be hazardous to your grade.
Procedure

1. Your Prep-Sep apparatus should already be put together for you. If not, use the diagram in Figure 1 to construct yours.

![Diagram of Prep-Sep assembly](attachment:prep-sep-diagram.png)

**Figure 1.** Prep-Sep® assembly, showing syringe, stopcock, and column.

2. The column must be conditioned to remove any contaminants and to wet the column. Pour approximately 5 mL of 100% isopropyl alcohol into the cone-shaped part of the apparatus – to the top of the sloped part. Turn the stopcock away from you (toward position A in Figure 1).

   Draw the isopropanol through the column slowly by gently pulling on the plunger of the syringe. Pull the liquid through the column until a small amount of liquid remains on top of the frit.

   Turn the lever on the stopcock up (toward position B in Figure 1) and discard the used isopropanol in the sink.

   **REMINDER:** When using this assembly, do not allow the column to go dry; that is, do not pull all the solvent through the column. Leave a small amount of the liquid on top of the frit. This technique requires that the column be wet at all times. And, once again, you must remember never to push on the withdrawing syringe – this will blow the column (and your chances of getting a good grade on this lab).

3. Once you have cleaned and conditioned the column, turn the stopcock to position A and draw about 5 mL of distilled water through the syringe to rinse the isopropanol off the column. Turn the stopcock to position B and discard the water into the sink.

4. Turn the stopcock on the syringe back to position A. Pour about 5 mL of one of the Kool-Aid solutions into the column (to the top of the sloped part). Draw the liquid through the column slowly and observe what happens to the column. Observe what happens to the column and record your observation in the chart on the data sheet.

   When you’ve finished drawing the liquid through the column (except for that little bit on top of the frit), turn the stopcock to position B and place some of this effluent in one of the wells of your well plate so you can keep up with the color of each extraction. Discard the remainder of the effluent in the sink.
5. Repeat Step 4 five more times, using approximately 5 mL of each of the following solutions in this order:

- Water
- 5% isopropanol
- 10% isopropanol
- 20% isopropanol
- 100% isopropanol

Record the required information for each extraction in the data table.

6. Wash the column with 5 mL of water and discard the effluent in the sink.

7. If time permits, repeat Steps 4 – 6 with a different Kool-Aid flavor.

**Disposal and Cleanup**

- Your instructor will want to look at the results of your extraction, so don’t empty your well plate before he or she has had a chance to see it.
- Once your instructor has checked your well plate, empty it into the sink and gently rinse it with tap water. Place it face down on a paper towel to dry.
- Pour any unused Kool-Aid solution down the sink and rinse the beaker with water. Do not pour it back into the large “stock” beaker.
- Place the syringe assembly on the lab bench. Lean it against something so that it remains in the upright position if possible.
- Wash your hands with soap and water before leaving the lab area.