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Edvo-Kit #
211

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Dangerous or Delicious: Using Chromatography to Examine Vaping

Experiment Objective:

Vaping is rising in popularity but many of its health effects are unknown. In this experiment, students become medical researchers and investigate the chemical contents of three simulated e-liquids using thin layer chromatography.

See page 3 for storage instructions.

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Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

	Components	Storage	Check ✓
A	Control	Freezer	❑
B	E-liquid Sample 1, "Banana Blast"	Freezer	❑
C	E-liquid Sample 2, "Cinnamon Swirl"	Freezer.	❑
D	E-liquid Sample 3, "Classic Clove"	Freezer	❑
E	Aqueous sodium citrate: isopropanol	Room Temp.	❑
F	Aqueous potassium acetate: ethanol	Room Temp.	❑

This experiment contains enough reagents for 10 lab groups.

Reagents and Supplies

- Thin Layer Cellulose Base Plates ❑
- Transfer pipets ❑
- Microcentrifuge tubes ❑
- 15 mL tubes ❑

Experiment Requirements *(NOT included with this experiment)*

- Pencils
- Rulers
- 250 mL beakers (6-7 cm in diameter)
- Gloves and safety goggles

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

None of the experiment components are derived from human sources.

Background Information

Smoking kills. The connection between smoking cigarettes and life-threatening conditions like heart disease, stroke, and lung cancer are clear and well known. But what about e-cigarettes and vaping? Here the messages are mixed. Sometimes e-cigarettes are presented as a safer alternative to more traditional tobacco products. Other times vaping is described as an alarming and dangerous new trend that's creating the next generation of nicotine users and causing lasting damage to people's lungs and bodies. Scientists in fields ranging from chemistry to cell biology are racing to learn more about vaping in order to resolve this dichotomy and provide confident, evidence-based health advice. In this lab, you will use chromatography to help tackle a key research question that underlies this work: what chemicals are vapers exposing themselves to?

BOX 1: Important Chemistry Terms

- **Vapor** - A gas formed by boiling or evaporating a liquid.
- **Aerosol** - A suspension of solid or liquid particles in a gas.
- **Solvent** - A material that easily dissolves other substances.
- **Absorbent** - A material that easily soaks up liquids.

EVERYTHING E - THE 101 ON VAPING

E-cigarettes come in countless shapes and sizes (Figure 1a) and go by many names including mods, vapes, tanks, e-hookahs, and ENDS (electronic nicotine delivery systems). Despite their diversity, all these devices have the same basic construction - a chamber to hold liquid, a heating element such as a coil, a battery, a microprocessor, and a mouthpiece (Figure 1b). Together, these parts heat solutions called e-liquids to between 100 - 250 °C (212 - 482 °F). This produces a smoke-like mist which is frequently referred to as a vapor, but is more accurately an aerosol. Many users breathe in this aerosol in order to deliver a potent dose of nicotine. Other users choose to consume nicotine-free e-liquids for the flavors, or to purchase or make black-market e-liquids that can contain additional drugs. Still other users avoid inhaling the aerosol altogether and instead use it to create intricate clouds of smoke. All these activities fall under the catch-all term of "vaping".

Figure 1: E-cigarettes are diverse but have similar core parts.

In recent years, the popularity of vaping has grown exponentially. Originally, vaping was promoted as a way for tobacco consumers to quit smoking. However today, many vapers have never used anything but ENDS and e-liquids. While vaping is considered less harmful than smoking cigarettes it is still not considered safe to use or to inhale second-hand.

Unfortunately, there are huge gaps in what we currently know about the safety and long-term health effects of vaping. Researchers are working to narrow these gaps through experiments and clinical tests rather than waiting to observe them in users. However, these researchers face several challenges. One is that many negative health effects may appear only after many years of repeated (chronic) use. Another is that the chemicals found in e-liquids and their aerosols are diverse and unpredictable.

"Perhaps we shouldn't be surprised that lung problems might develop in people who vape: our lungs were meant to inhale clean air and nothing else. It took many years to recognize the damage cigarettes can cause. We could be on a similar path with vaping."

Quote by Robert H. Shmerling, MD Senior Faculty Editor, Harvard Health Publishing

Despite these challenges, several health risks are beginning to emerge (Figure 2). Except for infrequent cases of ENDS units causing dangerous explosions or fires, most health concerns are related to the chemical components of e-liquids. These include solvents like propylene glycol, drugs like nicotine, and contaminants like heavy metals as well as a range of flavors, preservatives, and aromatic transporters.

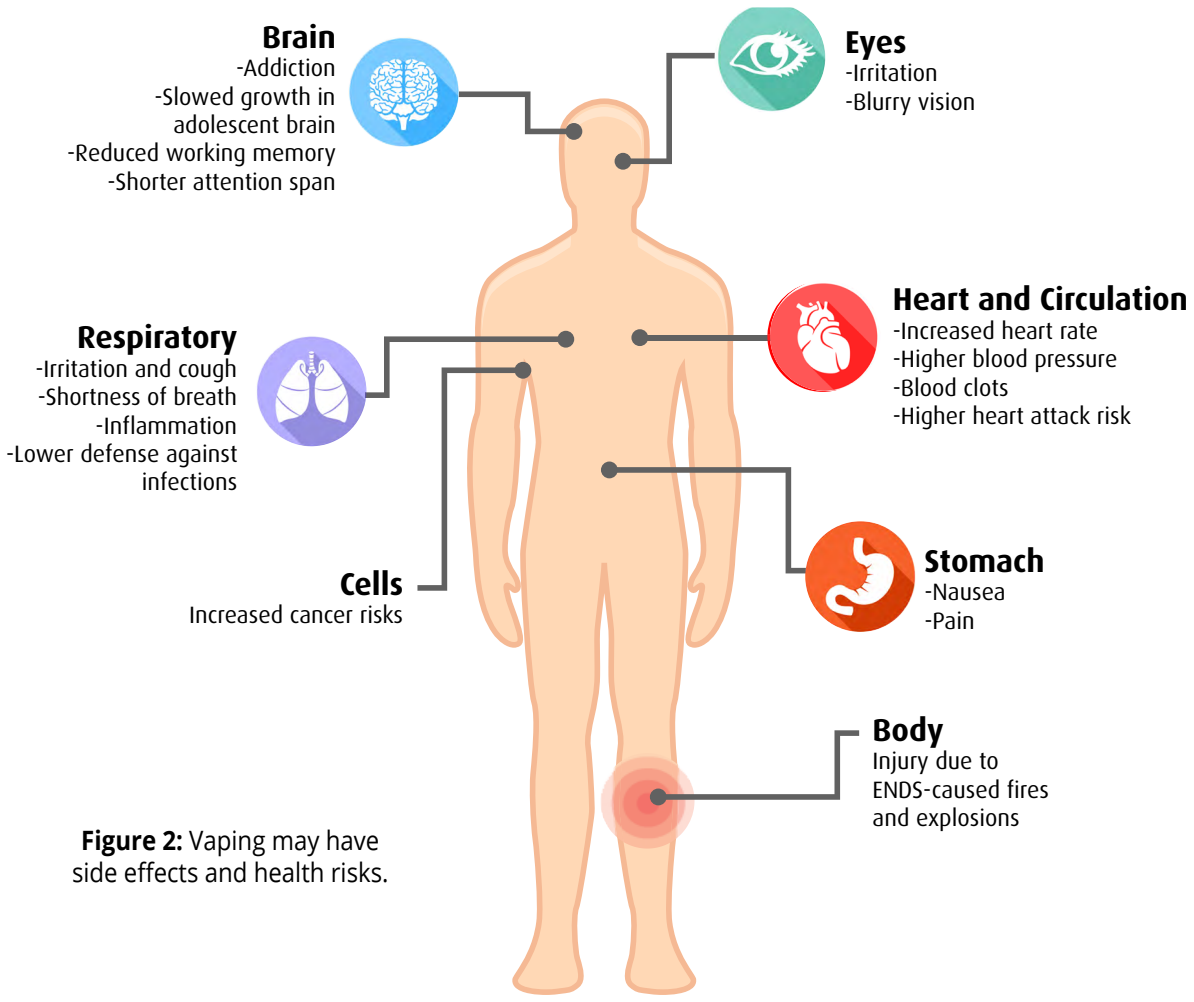


Figure 2: Vaping may have side effects and health risks.

THE CHEMICAL CONTENTS OF E-LIQUIDS

Scientists studying the chemical composition of e-liquids have identified around 100 different chemical ingredients and contaminants. Several of these substances are capable of causing cancer in living tissue and are categorized as carcinogens. Others are classified as irritants because they cause inflammation in our bodies or as poisons because they cause injury and death at high doses. Many more are compounds whose behavior at high temperatures and whose effects on human cells and organs are unknown.

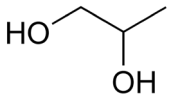


Figure 3a: Solvents like propylene glycol ($CH_3CH(OH)CH_2OH$) make up 90-95% of e-liquids.

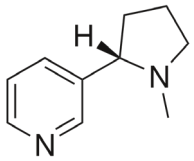


Figure 3b: Users can buy e-liquids with 0-5% nicotine. (Although tests have found nicotine even in e-liquids labeled "Nicotine-Free".)

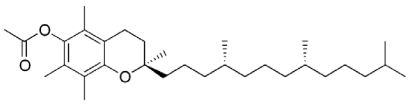


Figure 3c: Vitamin E acetate was the likely cause of the 2019 EVALI outbreak.

The primary ingredient in many e-liquids is propylene glycol (Figure 3a). This popular solvent dissolves, delivers, and preserves the other ingredients in an e-liquid and also makes the aerosol feel smoother. Propylene glycol is a colorless and odorless liquid, but it is by no means as safe as water. While it has low toxicity at room temperature, in the high heat environment of some ENDS it can form dangerous carbonyl compounds like formaldehyde and acetaldehyde. In response to this discovery, many e-liquid producers are now switching to ethylene glycol or glycerin-based solvents.

Another major ingredient in most e-liquids is synthetic or extracted nicotine (Figure 3b). Nicotine itself is not classified as a carcinogen. However, it can still affect a person's cardiovascular, respiratory, gastrointestinal, immune, and nervous systems. For example, the initial kick that nicotine provides is because it triggers the release of adrenaline and causes insulin resistance in cells. These effects briefly raise the user's blood sugar levels and their blood pressure which gives them a "buzzed" feeling. At the same time, these effects increase the user's risk of having a heart attack, experiencing multiple blood clots, or developing type-2 diabetes.

Nicotine's strongest effects are on the nervous system. In the brain, nicotine causes the release of dopamine which creates a temporary feeling of calm and contentment. However, this release also makes nicotine highly addictive. Stopping, decreasing, or even just maintaining regular nicotine use causes the brain to send strong "more" signals called cravings. Ignoring these signals can cause moodiness, lack of focus, irritability, depression, anxiety, and physical discomfort. These symptoms are temporary but serious because they cause users to continue to vape even when they'd like to quit or to supplement with traditional cigarettes. In addition, repeated nicotine use has been linked to slower brain development in young adults, disturbed sleep, and trouble concentrating.

What about the other 98+ potential chemicals? Many are intentionally included compounds used to add flavor, transport a certain aroma, or help preserve the solution. These additives vary tremendously between different brands and types of e-liquids but most meet food quality standards. However, the high heat of most vaping devices can also cause these chemicals to change or react with one another and create new compounds. Also, the health effects of many chemicals are different when inhaled rather than ingested. Consequently, ingredients that are labeled safe for consumption can still be dangerous when aerosolized and inhaled. A striking example of this is the EVALI epidemic.

> "It's unacceptable that a user of e-cigarettes is being told by the cigarette industry that its products are safe and at the same time they are breathing in these toxic chemicals."
>
> *Erika Sward, American Lung Association*

In the late summer of 2019, there was an outbreak of severe lung infections in young and otherwise healthy individuals. A common denominator was that all sufferers were vapers, leading to the condition being named EVALI (E-Cigarette or Vaping product use Associated Lung Injury). Investigators found vitamin E acetate (Figure 3c) in the lungs of EVALI patients but not in healthy individuals. This synthetic form of vitamin E is used routinely in nutritional supplements and skin creams and was added to several black market e-liquids as a "safe" thickener. However, when it was inhaled it severely

damaged the user's lungs and caused them to experience shortness of breath and chest pains. EVALI eventually caused thousands of hospitalizations and 55 reported deaths.

BOX 2: Example Contaminants

Acrolein	Nickel
Acetic Acid	Nicotine
Acetone	N-Nitrosonornicotine
Benzene	Phenanthrene
Cadmium	Pesticides
Isoprene	Toluene
Lead	Tin
Mercury	Xylene

Finally, e-liquids contain contaminants. These chemicals are often present at very low concentrations of less than 1% but can still be toxic and cancer-causing. Contaminants can come from manufacturing processes like extraction and synthesis, from the pesticides and herbicides that are used to grow certain ingredients, and from a user's own vaping device. For example, some heating coils degrade following repeated use and release trace amounts of heavy metals into the aerosol. Box 2 provides a shortlist of contaminants that have been found repeatedly in e-liquids. Detecting these chemicals is challenging because contamination occurs sporadically and because contaminants are usually present in low and hard to detect amounts. Luckily, several technologies help investigators screen for impurities. These technologies are also being used to monitor the changing contents of e-liquids and to identify key chemicals for clinical studies.

CHROMATOGRAPHY

"What's in this?" is a deceptively simple question that takes ingenuity, expertise, and hard work to answer. Analytical chemists tackle this question by separating, identifying, and measuring matter using laboratory techniques like chromatography. In chromatography, the components of a mixture are dissolved in a specially chosen liquid or gas (known as the mobile phase) and then carried through a solid, porous media (known as the stationary phase) by gravity, capillary action, or an external current. During this process, different molecules move at different speeds based on their ability to dissolve in the mobile phase and to adhere to the stationary phase (see **Did you Know?**). This causes these different sub-parts of the mixture to become physically separated and enables scientists to identify, measure, or extract them.

DID YOU KNOW?

- A molecule with HIGH ADHERENCE to the stationary phase will move *slowly*.
- A molecule with LOW ADHERENCE to the stationary phase will move *quickly*.
- A molecule with HIGH SOLUBILITY in the mobile phase will move *quickly*.
- A molecule with LOW SOLUBILITY in the mobile phase will move *slowly*.

There are many different chromatography techniques (Figure 4). One of the simplest and most accessible is paper chromatography, where a strip of paper with a dot or line of ink is partially submerged in a small amount of water or alcohol. As the water or alcohol (the mobile phase) moves up the paper (the stationary phase) it causes the ink to dissolve, spread, and separate into its different colors. At the other end of the spectrum is gas chromatography paired with mass spectrometry (GC-MS). This is the gold standard and workhorse of modern analytical chemistry. In this procedure, a mixture is first dissolved in a gas like helium or nitrogen and then passed through a glass column coated with a stationary phase. Next, as different chemicals migrate out of the column, their molecules are converted into ions so that scientists can calculate each

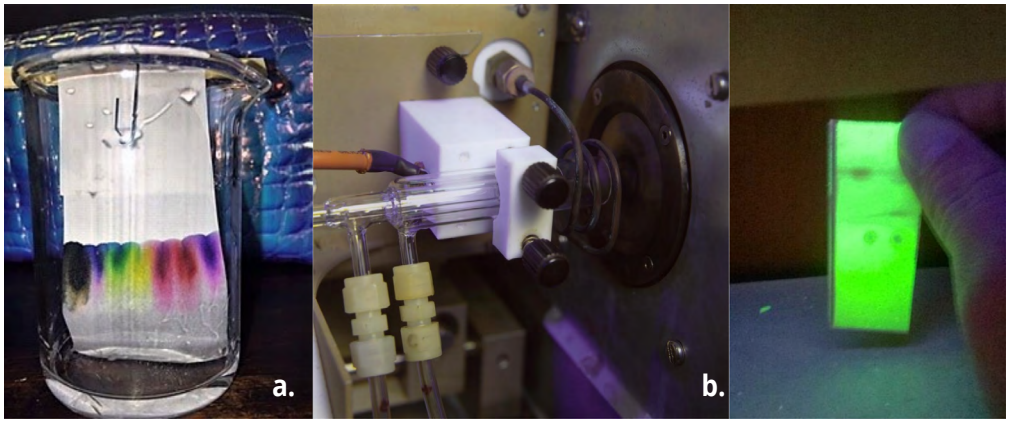


Figure 4: Popular chromatography methods: (a) paper chromatography, (b) gas chromatography with mass spectrometry, (c) thin layer chromatography.

From Wikimedia Commons: Paper chromatography in progress.jpg by Amitchell125

IMAGE 408 001 007 courtesy US Department of Energy (Flickr Photostream)

From Wikimedia Commons: Tlc plate.jpg by Tiepcmaniac

chemical's mass to charge ratio. In this lab, you will be using thin-layer chromatography (TLC) which combines elements of both paper and gas chromatography.

In thin-layer chromatography, the stationary phase, also known as the absorbent, is plated onto a glass or plastic plate to create an even, thin (<0.1 mm thick), and sturdy matrix. A small amount of each sample is then applied directly to this plate and allowed to dry. Next, the plate is placed in a beaker containing a solvent. At this stage, only the edge of the plate nearest the sample is in contact with the solvent. However, because the absorbent is dry, the solvent is drawn up the plate. The sub-parts of a sample also move up the plate along with the solvent but at different rates. Before the solvent reaches the top of the plate, it is removed, dried and, in many cases, stained. The experimenter then analyzes the resulting spot pattern and often calculates a retardation factor (Rf) for each separated component. This is done by dividing the distance a component traveled up the plate by the total distance traveled by the solvent.

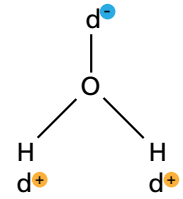


Figure 5: A water molecule. Its oxygen atom has a positive charge and its hydrogen atoms have a negative charge although the overall molecule is electrically neutral.

Thin-layer chromatography is particularly sensitive to small differences in the structure and polarity of different chemicals. Structural features include molecular weights, geometry, and carbon-carbon double bonds. These physical traits influence how easily a molecule migrates through the stationary phase. Polarity describes molecules that have sub-areas of positive and negative charge. This occurs when one nucleus in a molecule attracts the negatively charged electron more strongly than the other nuclei (Figure 5). Polarity directly affects solubility because polar substances dissolve better in polar solvents and non-polar substances dissolve better in non-polar solvents (Figure 6).

The stationary phases used in TLC experiments are usually a mixture of neutral molecules, polar molecules, and even more strongly charged ionic compounds. These molecules interact with polar molecules in a sample and slow their rate of migration. By selecting different mobile and stationary phases scientists can isolate a wide range of chemicals. This allows TLC to be used in many settings ranging from crime-solving to environmental safety tests to medical research. In today's lab, you will be using TLC to examine the chemical contents of three flavored e-liquids.

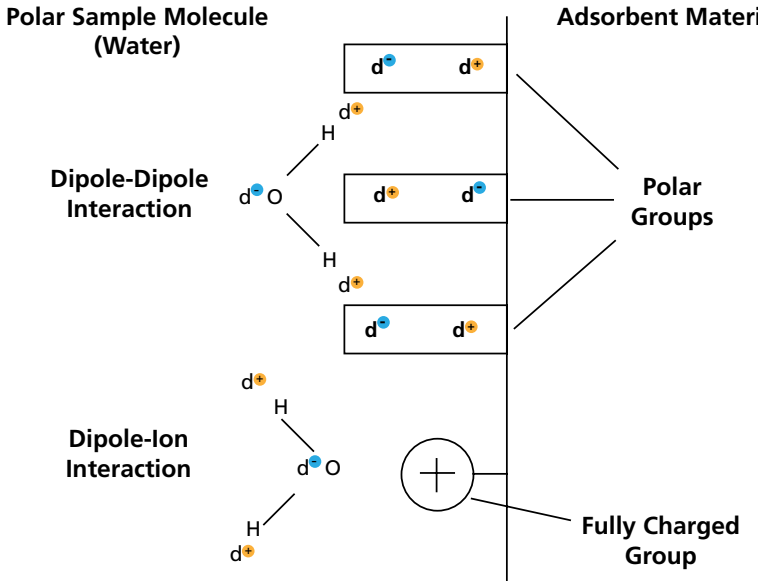


Figure 6: Molecules in a sample are attracted to oppositely charged molecules in the absorbent and solvent and form dipole-dipole and dipole-ions interactions.

Experiment Overview

EXPERIMENT OBJECTIVE

Vaping is rising in popularity but many of its health effects are unknown. In this experiment, students become medical researchers and investigate the chemical contents of three simulated e-liquids using thin layer chromatography.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

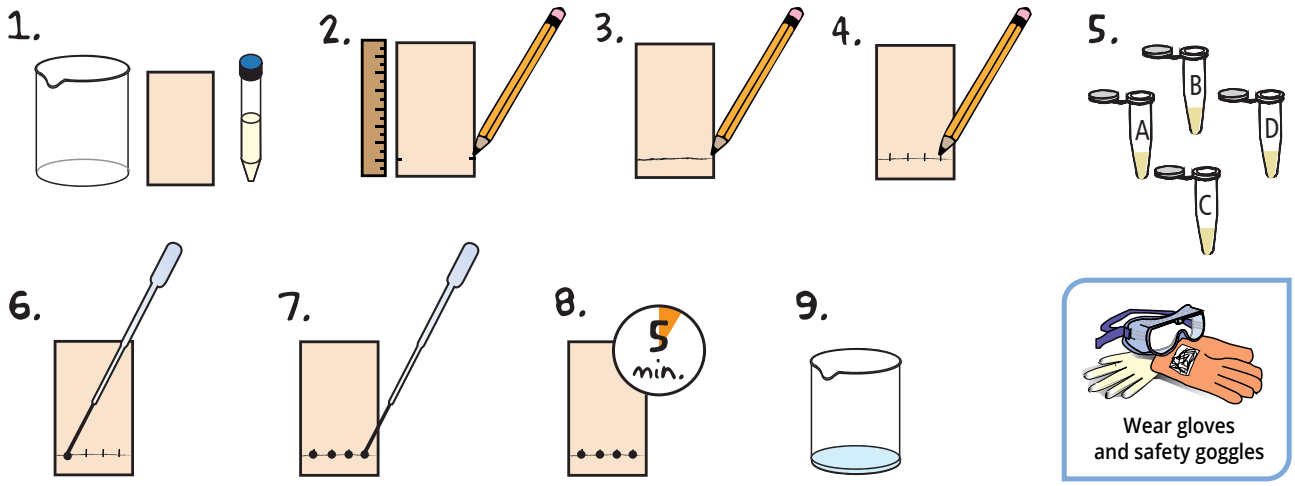
During the Experiment:

- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Thin Layer Chromatography



1. **OBTAIN** a TLC plate, beaker, and 15 mL tube of solvent from your instructor. Record the name of the solvent here: _____.
2. **ORIENT** the plate so that the shorter edge is horizontal and the longer edge is vertical. Using a ruler and blunt pencil, **MEASURE** 1 cm from the bottom and gently mark both sides at this height.
   NOTE: For steps 2, 3 and 4 make sure you use a regular pencil, NOT a pen or colored pencil as the colors in these will obscure the results.
3. Very gently **DRAW** a straight line across the plate using the two 1 cm marks. This will serve as the origin line.
   NOTE: The absorbent can be easily displaced during this step. Write VERY SOFTLY on the plate and take your time.
4. **DIVIDE** and gently mark the origin line into 4 spots starting ~0.5 cm from the edge and leaving ~0.5 cm between each spot.
5. **COLLECT** the control and three e-liquid samples (tubes A-D) from your teacher. These may be in snap-top tubes made specifically for your group or in shared classroom tubes. **OPEN** each tube and smell the samples to confirm that the scent of each e-liquid matches it's flavor description. The control will have no scent.
6. Use a transfer pipet to **TRANSFER** 1 µL of the control to the first spot (see **IMPORTANT NOTE**).
7. **REPEAT** step 5 for the e-liquid samples 1, 2, and 3.
8. Let the sample spots **DRY** at room temperature for 5 minutes.
9. **ADD** 4-5 mL of solvent to a beaker. The solvent's depth will vary depending on the beaker's size but should be between 0.5-0.9 cm. If the solvents depth is more than 0.9 cm, **REMOVE** an appropriate amount before continuing. If the solvent sits below 0.5 cm, add more until it reaches that height.

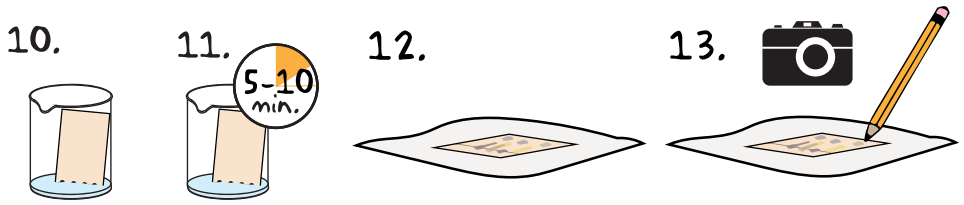
continued

IMPORTANT NOTE (Step 6):

Very little sample is needed to achieve results. The smaller the diameter of the spot the easier the final separation will be to read. If you are using a plastic mini pipet minimize the amount of sample applied by: (1) Placing the end of the pipet just below the surface of the dye in the microtest tube and gently squeezing the bulb to allow a small amount of liquid to fill the very tip. (2) Holding the pipet vertically and touching the tip to the plate above the appropriate spot. (3) Allowing a small amount of dye to flow out and onto the adsorbent.*

**If this happens, quickly lift the pipet away. If this does not happen, very slightly squeeze the bulb and then lift the pipet away.*

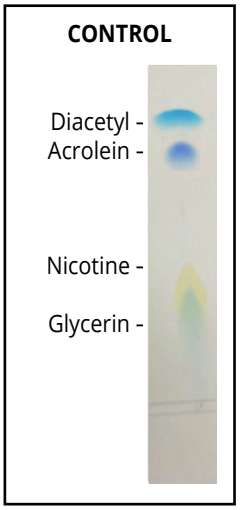
Thin Layer Chromatography, continued



10. Carefully **PLACE** the bottom edge of your plate into the beaker and lean it against the side so that it remains upright.
12. **INCUBATE** at room temperature for 5-10 minutes. During this time the solvent should move up the TLC plate but not reach the top.
12. Before the solvent reaches the top edge, **REMOVE** the TLC plate and lay it flat on a paper towel with the absorbent side up.
13. **MARK** the edge of the solvent front with the pencil and **TAKE** a picture.
14. **COMPARE** the pattern of each sample with the control sample. What chemicals are present in each of the samples?
15. (Optional) **CALCULATE** the Rf for each chemical in the control.
    a. **MEASURE** the distance that the solvent front traveled from the origin line. **RECORD** this value in Table 1.
    b. **MEASURE** the distance that the four known sub-components traveled from the origin points. **RECORD** these values in Table 1.
    NOTE: The spot created by a chemical can vary in sizes and shape depending on how much sample was applied to the plate, the concentration of the chemical in the sample, and the chemical's nature. When identifying how far a compound travels, measure from the center of the spot.
    c. **CALCULATE** the Rf value of each component and **RECORD** these values in Table 1. The Rf value is the distance traveled by a sample from its origin divided by the distance that the solvent traveled.
16. (Optional) **REPEAT** step 15 for e-liquid samples 1, 2, and 3.

TABLE 1

	Distance Travelled	Rf
Solvent		N/A
Diacetyl (top, light blue spot)		
Acrolein (middle, dark blue spot)		
Nicotine (middle, yellow spot)		
Glycerin (bottom, blue spot)		



Study Questions

1. Describe at least two challenges that researchers face when trying to determine the health risks of vaping.

2. Why might a substance that is classified as safe for consumption be dangerous when in an e-liquid?

3. What does EVALI stand for? Do you think that an outbreak, similar to the summer 2019 outbreak, could occur again? Why or why not?

4. Select a chemical that has been found in e-liquids (you can use any mentioned in the background, in Box 2, in the experimental control ladder, etc.) and find out three interesting facts about it. Next, list two additional things that you would like to know about this chemical if you were about to consume it.

5. What are the basic principles of Thin Layer Chromatography?

Instructor's Guide

NOTES TO THE INSTRUCTOR

This lab is designed for 10 lab groups. Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

What to do:	When:	Time Required:
Prepare TLC Plates	Anytime before experiment.	10 min.
Prepare Samples and Solvent	Day before or day of the experiment.	15 min.

Pre-Lab Preparations

Prepare TLC Plates

1. Handle the plate by its edges. **DIVIDE** the plate into ten (10) 4 x 5 cm squares by lightly drawing a line using a blunt pencil and the straight edge of a ruler on the cellulose adsorbent.

2. Carefully **CUT** the plate into ten pieces with a sharp pair of scissors. Do not use a paper cutter.

Prepare Samples and Solvent

3. **THAW** Components A-D at room temperature for 5-10 minutes.

4. During storage, the dyes used in Components A-D may have precipitated out of the solution. If this has happened, shake, tap on a hard surface, or use a vortex to **RE-MIX** these liquids.

5. Either allow each group to share tubes A, B, C, and D or **ALIQUOT** 10-12 µL to ten small tubes for each group.

6. **ALIQUOT** 5 mL of aqueous sodium citrate: isopropanol (Component E) to ten* conical tubes.
   ** Or five if your class is doing the optional solvent activity (see below).*

For this experiment, each group should receive:

- 1 small beaker
- 1 tube of solvent
- 4 transfer pipets or 1 adjustable micropipette and tips* (Preferred)
- 4 dye samples
- Ruler
- Blunt Pencil

** While a 1-10 µL adjustable pipette will allow students to measure exactly 1 µL, any adjustable pipette will provide your students with more control when spotting small amounts of the samples onto the TLC plate.*

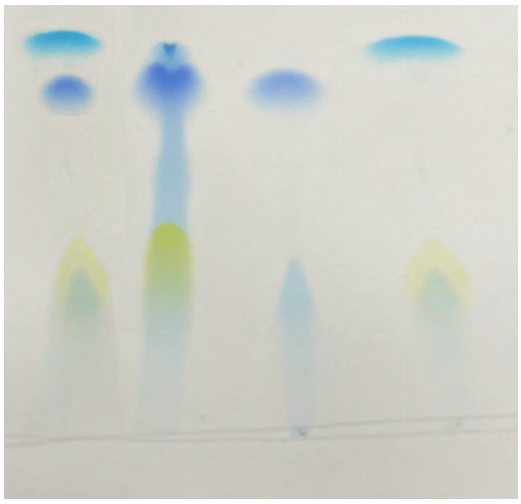
OPTIONAL SOLVENT ACTIVITY

The composition of the solvent used in a TLC experiment can be varied to provide a virtually unlimited set of conditions for chromatography. These different conditions help scientists separate and identify all chemicals in a sample. To demonstrate this, we have included an additional solvent: aqueous potassium acetate:ethanol (Component F).

If you decide to incorporate this solvent into your experiment:

7. **ALIQUOT** 5 mL of aqueous potassium acetate:ethanol (Component F) to five tubes.

Expected Results and Analysis



Control | E-liquid #1 "Banana Blast" | E-liquid #2 "Cinnamon Swirl" | E-liquid #3 "Classic Clove"

E-liquid	Name	Solvent(s)
#1	"Banana Blast"	Acrolein, Nicotine, Glycerin
#2	"Cinnamon Swirl"	Acrolein, Glycerin
#3	"Classic Clove"	Diacetyl, Nicotine, Glycerin

TABLE 1 (Using aqueous sodium citrate: isopropanol)

	Distance Travelled	Rf
Solvent	4 cm	N/A
Diacetyl (top, light blue spot)	3.7 cm	0.92
Acrolein (middle, dark blue spot)	3.3 cm	0.825
Nicotine (middle, yellow spot)	2.0 cm	0.500
Glycerin (bottom, blue spot)	1.8 cm	0.450

Optional Solvent Activity

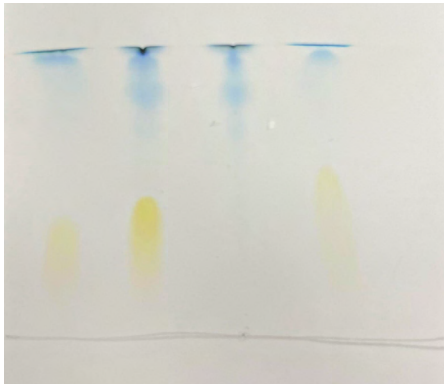


TABLE 2 (Using aqueous potassium acetate:ethanol)

	Distance Travelled	Rf
Solvent	4 cm	N/A
Diacetyl (top, light blue spot)	4 cm	1
Glycerin (middle, blue spot)	3.9 cm	0.975
Nicotine (bottom, yellow spot)	1.6 cm	0.400

Please refer to the kit insert for the Answers to Study Questions