Sowing the Seeds of Neuroscience

Chromatography:

Observing Complex Combinations

of Chemical Compounds

Chromatography: Observing Complex Combinations of Chemical Compounds

Activity Time: Two 50 minute periods, with additional time for homework assignments.

Lesson Summary:

In this lesson, students learn that plant extracts are mixtures of many chemical compounds and it is possible to separate these compounds using chromatography. The health benefits of a medicinal plant may be due to one compound or to a combination of chemical compounds working together. In the lab portion, students will do chromatography of a black marker to understand how a single liquid (black ink) is comprised of many different chemicals. They will also do chromatography with plant material to see if multiple components can be separated.

STUDENT UNDERSTANDINGS

Big Idea & Enduring Understanding

- **Chromatography:** Plant extracts are mixtures of many compounds. It is possible to separate these compounds using chromatography.

Essential Question

- Do plant extracts separate into different compounds that I can see using chromatography?

Neuroscience Core Concepts

- The plant world is filled with species that contain chemicals with medical properties, including neuroactive properties.
- Traditional healers around the world use plants to treat illnesses, including neurological and mental illness.
- There are many people with mental and neurological disorders in our society.
- Plant extracts may contain chemicals which can treat neurological and mental illnesses.

Learning Objectives

Students will know…

- The plant world is filled with species that contain chemicals with medicinal properties, including neuroactive properties.
- Plant extracts are composed of multiple chemicals.
- Chromatography is a method to separate the components of a complex mixture.
- A plant's health benefits may be due to one chemical or to a combination of chemicals.

Students will be able to…

- Demonstrate lab safety procedures.
- Follow the procedures to successfully conduct a paper chromatography of a plant.
- Record laboratory data.

Standards Alignment

Washington State Essential Academic Learning Requirements (EALRs): Science
Science EALR 2: Inquiry • 6-8 INQB—Investigate • 6-8 INQE—Model Science EALR 3: Application • 6-8 APPG Science EALR 4: Physical Science • 6-8 PS2A • 6-8 PS2B
Common Core Standards: English Language Arts (ELA)
Reading Standard for Literacy in Science and Technical Subjects: Key Ideas & Details • CCSS.ELA-Literacy.RST.6-8.3 Writing Standard for Literacy in Science and Technical Subjects: Text Types & Purposes • CCSS.ELA-Literacy.WHST.6-8.2d Language Standard 4c & 6: Vocabulary Acquisition & Use • CCSS.ELA-Literacy.L.6.4c • CCSS.ELA-Literacy.L.6.6

TEACHER PREPARATION

Materials

Classroom Materials

Item	Quantity
Student lab notebook	1 per student
Pen and pencil	1 per student
Copies of *Chromatography Lab Background Reading—Student Handout*	1 per student
Copies of *Chromatography Lab Procedure—Student Handout*	1 per student
Copies of *Chromatography Data—Student Handout*	1 per student
Copies of *Chromatography Results & Conclusions—Student Handout*	1 per student
Optional: Copies of *Chromatography Vocabulary Quiz—Student Handout*	1 per student

Laboratory Materials

Review the materials with your students. It will be helpful to show them each piece of lab equipment and mention how it will be used in this activity.

Most lab materials are provided in the *Sowing the Seeds of Neuroscience* classroom kit. However, fresh vegetables, fruits, flowers, and medicinal plants are not included. We highly recommend that you purchase some red cabbage and spinach for this lesson; in addition, encourage your students to bring in other vegetables, fruits, flowers, or fresh medicinal plants.

The starred (*) items below are not provided. There are enough materials in the classroom kits for ten groups to do this laboratory. Depending on class size, groups should be made of two to three students.

Item	Quantity
Spinach, purple cabbage and other vegetables, fruits, flowers or medicinal herbs*	1 per group
Plastic pipettes	1 per group
Filter paper strips	3 per group
Parafilm	3 squares per group
Scissors*	1 per group
Water	About 30 ml per class, you must aliquot into test tubes
Ethanol	1 bottle per school, you must aliquot into test tubes
Acetone	1 bottle per school, you must aliquot into test tubes
Pencils*	1 per group

Penny or other coin*	1 per group
Black Sharpie flip chart marker	1 per group
Test tubes with solvent	3 per group
Test tube rack	1 per group
Lab tape	1 per group
Timer	1 per group
Mortar and pestle	1 per group
Metric ruler	1 per group

Safety Materials

Item	Quantity
Safety goggles*	1 per student
Gloves*	1 pair per student

*Safety materials are provided in the classroom kit only if you requested them.

Lab Safety

- Students must not eat or drink anything in the lab.
- Students must wear safety goggles and gloves.

Preparation

- Photocopy Student Handouts.
- Assign the *Chromatography Lab Background Reading—Student Handout* to students as homework.
- Purchase fresh spinach and purple cabbage. In addition, encourage your students to bring in vegetables, fruits, flowers, or fresh medicinal plants.
- Aliquot water, acetone, and ethanol for students to use. Pipette 1 ml of water into 10 tubes, 1 ml of acetone into 10 tubes, and 1 ml of ethanol into 10 tubes. Each student group should receive three test tubes. We recommend that each group has only ***one*** solvent to test—so that each group gets three test tubes with the same solvent. This will allow the class to compare results with the same plant and different solvents. Once filled, test tubes should be covered with parafilm.
- Plan your timing.

- On Day One, introduce chromatography using the *Engage* activities, have students read the background reading if they haven't already done so, and prepare strips by following the *Day One* laboratory procedures. This will give the strips plenty of time to dry before running.

- On Day Two, students can follow the *Day Two* laboratory procedures to run and dry the strips, record information in their lab notebooks, and participate in the Chromatography Obstacle Course. Deliver the *Explain*, *Elaborate*, and *Evaluate* activities. While students wait for the strips to run, you may want to engage them in a review of the lesson's vocabulary or ask them to read the background reading for the next day's lab activity from the *Sowing the Seeds of Neuroscience* curriculum.

TEACHER PROCEDURE

Day One

Engage

1. Explain the purpose of the lesson and review Big Idea, Enduring Understanding, and Essential Question.

2. Review the *Depression Hurts* and *An Uplifting Plant with a Strange Name* sections of the background reading. This example shows how a plant, such as St. John's wort, may have chemical compounds in it that can be used to treat depression, a neurological disorder. Pharmacologists are challenged to determine what specific neuroactive chemicals in the plant, or what combination of chemicals, work together to treat depression. They need to identify what chemical compounds are present in the plant, which one(s) have the intended effect, and how they work on the nervous system.

3. Hold up a spinach leaf and a similarly-sized purple cabbage leaf. Tell students to suppose that in an indigenous community, a traditional remedy for treating the symptoms of seizures and sadness (which we know are caused by the neurological disorders epilepsy and depression) is made using either of these plants. Don't tell students the names of the plants.

4. Tell students that for the next two days, they are pharmacologists (scientists who develop drugs) wanting to determine what chemical is the active compound in these two plants that acts on the nervous system. You know that each plant probably has many different chemicals, some which might be neuroactive. Knowing the number of compounds in each plant will help you to further plan your research and future experiments.

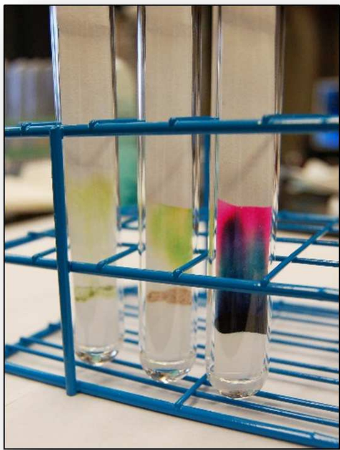
5. Reflect back on the *Chromatography: Combinations of Complex Components* section of the student reading. Ask students to discuss:

- What does chromatography do?
- How is chromatography useful to us as pharmacologists?

Explore

6. Review lab safety information with students. Remind students to put on their gloves and safety goggles. Tell students not to taste any of the plants they will be working with today.

7. Hand out copies of the *Chromatography Lab Procedures—Student Handout*, one per student.



8. Tell students that today they will prepare their strips and allow them to dry overnight. Tomorrow, they will run their strips.

9. Allow time for student groups to prepare their strips by following the *Day One* procedures outlined on the Student Handout.

Day Two

10. Distribute copies of the *Chromatography Data—Student Handout*, one per student.

11. Allow time for student groups to complete the investigation, following the *Day Two* procedures outlined on the *Chromatography Lab Procedures—Student Handout.*

12. When all groups have completed the lab, provide instructions on how you would like students to clean up their lab stations and put away lab equipment.

13. Distribute copies of the *Chromatography Results & Conclusions—Student Handout*, one per student. Allow time for students to work together with their group members to answer the questions.

    Note: The marker ink chromatography that each group does allows for all student groups to have a "reference standard". When discussing the results of their specific plant extract and solvents, each group can talk about their bands in comparison to the marker reference standard.

Explain

14. Review the *Chromatography: Combinations of Complex Components* section of the background reading. Ask students, what is one of the reasons why chromatography works?

    From the Student Handout: "One reason why chromatography works is because different chemical compounds in the extract are different sizes. When these molecules dissolve in the **solvent** (water, acetone, or ethanol) and travel up the filter paper, small molecules travel faster and large molecules travel slower because they literally bump into the weave of the filter paper more often."

15. Ask students to participate in a **Chromatography Obstacle Course**, which will model how small and large molecules travel up the filter paper. To create the course, ask all of the students in the class, except three volunteers, to stand close together. You might need to go out into the hallway or outside to have enough room. Students should stand clustered together, with just enough space in between them that a person could squeeze through.

16. Explain that the paper is like an obstacle course (a tangled web of cellulose fibers, representing the solid, stationary phase in chromatography). The solvent wicks up the filter paper, carrying with it the molecules in the plant extract or marker ink (the mobile, liquid phase in chromatography).

17. Next, challenge the student volunteers to work their way through the obstacle course. First, send a single student through the course, trying to work his or her way from one end of the "filter paper" to the other, squeezing and weaving between the students as quickly as possible. Single students represent small molecules—and they will get through the maze relatively fast.

18. Next, send two or three students, with linked elbows, through the course. Pairs or triplets of students represent larger molecules—they will have a much harder time getting through the obstacle course because they will bump into more students. This models how larger molecules will take longer to travel up the filter paper.

19. **What is going on with this obstacle course model?** Explain that the molecules within the plant or ink are traveling a distance according to their attraction to the paper relative to their attraction to the solvent. In paper chromatography with filter paper, small molecules will travel

more quickly than large molecules. (You will see the difference in the distance traveled when comparing the same plant extract but using two different solvents).

This is true with our specific situation: when conducting paper chromatography with filter paper, large molecules travel more slowly than small molecules up the filter paper. However, with other types of chromatography, the opposite may be true.

Elaborate

20. Compare the class data. What similarities/differences occurred in the data between groups that conducted chromatography on the same plants? What were the similarities/differences between the three solvents on the same plant? What were the similarities/differences between the plants and ink?

21. What will students likely see on their filter strips? **They will see a series of colorful bands**.

    - **On the plant strips**, they will likely see green and yellow bands. Green plants like spinach have an abundance of chlorophyll, the green pigment that is necessary for photosynthesis. Purple or red plants like purple cabbage have chlorophyll (green/yellow bands) as well as anthocyanins, which are red/purple pigments (red/purple/pink bands). These colorful bands demonstrate that there is a complex combination of chemicals within each plant, which makes it a difficult task to discover the specific chemical or combination of chemicals that has the neuroactive effect.

    - **On the marker strips**, they will likely see a variety of colorful bands. The black ink in the marker is made up of a variety of colors of pigment. The chromatography process separates out the different colored pigments.

22. **What does the number of bands represent?** Each band represents ***at least one*** distinct chemical, but each band may include ***multiple*** chemicals that all traveled the same distance up the filter paper. The number of bands tells you a minimum number of chemical compounds in each plant, although the actual number might be larger.

23. Refer back to the *Depression Hurts* and *An Uplifting Plant with a Strange Name* sections of the background reading. Ask students to discuss in what ways chromatography might be useful for pharmacologists who are trying to determine the neuroactive properties in St. John’s wort and other plants that may be used to treat depression.

Evaluate

24. As a class, discuss students' responses to the prompts on the *Chromatography Results & Conclusions—Student Handout.* A scoring guide has been provided for this learning task.

25. ***Optional:*** Assign a vocabulary quiz using the terms introduced on the Student Handouts. Hand out copies of *Chromatography: Vocabulary Quiz—Student Handout* and administer it like a quiz, with no peeking at lab notebooks or the Background Reading. An answer key for the crossword puzzle is provided in the *Scoring Guides* section of this lesson plan.

SCORING GUIDES

Answer Key for Vocabulary Quiz

11 possible points.

Across
2. Chromatograph
5. Solvent
6. Mortar
7. Pestle
8. Chromatography
11. Chromatogram

Down
1. Bioactive chemical
3. Plant extract
4. Neurotransmitter
5. Serotonin
9. Neuroactive
10. Filter paper

Scoring Rubric for Results & Conclusions Student Handout
25 possible points.

Results

Dimension	Needs Work (0 Points)	Basic (1 Point)	Proficient (3 Points)	Advanced (5 Points)
Question #1: Explain why you see the different chemical compounds separate out as different color lines or smears on the filter paper.	Demonstrates an incorrect understanding of chromatography.	Explains that different chemical compounds in the extract/ink are different sizes.	Explains that different chemical compounds in the extract/ink are different sizes and travel at different speeds up the filter paper.	Explains that different chemical compounds in the extract/ink are different sizes. When these molecules dissolve in the solvent and travel up the filter paper, small molecules travel faster and large molecules travel slower because they literally bump into the weave of the filter paper more often.
Question #2: How many bands can you see in your marker chromatography? Therefore, what does this tell you about the minimum number of chemical compounds that are in this ink? Why do you think there was a difference between the plant extract and the ink?	Doesn't provide the number of bands on their marker strip.	Provides the number of bands on their marker strip.	Provides the number of bands on their marker strip. Correctly explains that each band represents at least one distinct chemical. Offers an idea for the different number of bands between the plant extract and the ink.	Provides the number of bands on their marker strip. Correctly explains that each band represents at least one distinct chemical, but each band may include multiple chemicals that all traveled the same distance up the filter paper. Offers an idea for the different

				number of bands between the plant extract and the ink.

Conclusions

Dimension	Needs Work (0 Points)	Basic (1 Point)	Proficient (3 Points)	Advanced (5 Points)
Question #3: Explain how chromatography is important in understanding the properties of medicinal plants.	Demonstrates an incorrect understanding of how chromatography is important to understanding the properties of medicinal plants.	Explains that chromatography is used to separate the components of a complex mixture.	Explains that chromatography is used to separate the components of a complex mixture. After you separate the components, you can isolate the different chemicals found in the plant.	Explains that chromatography is used to separate the components of a complex mixture. After you separate the components, you can isolate the different chemicals found in the plant and determine: A) what those chemicals are; and B) what bioactive and neuroactive effects they have.
Question #4: After the class has had a chance to compare the results of the different groups, answer this question: Which solvent was most effective at separating compounds? Why do you think this might be?	Doesn't identify a solvent.	Identifies either water, acetone, or ethanol as the most effect solvent.	Identifies either water, acetone, or ethanol as the most effect solvent. Supports this choice with class data.	Identifies either water, acetone, or ethanol as the most effect solvent. Supports this choice with class data. Offers an idea for why this solvent might be most effective at separating the chemical compounds.

EXTENSIONS

Take your students on a field trip to the University of Washington's Medicinal Herb Garden. Free docent-guided tours are available year-round, focusing on medicinal properties of many plants, historical uses of Pacific Northwest native plants, and the origin of many medicines still used today. To schedule a tour to the Medicinal Herb Garden or the UW Botany Greenhouse, visit this link: http://www.biology.washington.edu/greenhouse/k12.html. While you are on campus, also check out the Erna Gunther Ethnobotanical Garden outside the Burke Museum of Natural History featuring plants used for medicine, food, and raw materials for Native Americans of the Pacific Northwest. Learn more about these UW gardens at these links:

About the UW Medicinal Herb Garden
http://www.biology.washington.edu/mhg/index.html

Photo Tour of UW Medicinal Herb Garden
http://nnlm.gov/pnr/uwmhg/

Erna Gunther Ethnobotanical Garden at the Burke Museum
http://www.burkemuseum.org/exhibits/browse/ethnobotanical_garden

TEACHER BACKGROUND & RESOURCES

Resources

Chromatography
Sowing the Seeds of Neuroscience
http://www.neuroseeds.org/links/chromatography

Chromatography in Food Production
Bio-Rad
http://www.bio-rad.com/en-us/applications-technologies/chromatography-food-production

Paper Chromatography in Forensics
The Gist, Glasgow Insight into Science & Technology
http://the-gist.org/2011/07/tlc-the-forensic-way/

"Forensic Ink Analysis" Video (9:02 mins)
Centre for Forensic Science, University of Strathclyde
http://www.youtube.com/watch?v=I34tz5nIPCs

Career Connections

Botanist: A biologist specializing in plant science. Botanists study more than 400,000 species of living organisms, from tiny algae to giant trees.

Chemist: A scientist specializing in chemical science. A chemist studies the properties of matter. Biochemistry, a specialty within the field of chemistry, is focused on the chemical processes that occur among living organisms, such as plants.

Ethnobotanist: A biologist that studies the relationships between plants and people, in particular how people from different cultures use plants in daily life.

Herbalist: A practitioner of herbalism, which is the study and use of medicinal plants.

Naturopathic doctor: A doctor of naturopathic medicine (N.D.) is a medical doctor that uses alternative medical treatments (including medicinal plants and herbs) in partnership with traditional medical treatments. While a N.D. may use prescription medication with a patient, they prefer to use natural healing agents.

Pharmacist: A healthcare practitioner with a specialty in pharmaceuticals and the safe use of these medications and therapies. Pharmacists are trained in understanding the biochemical mechanisms of drugs and their interactions with the human body. In the United States, a pharmacist must have a Doctor of Pharmacy (Pharm.D.) degree and licensure.

Pharmacologist: A biomedical scientist that studies the interactions between drugs and cells, tissues, organs, or entire organisms. Pharmacologists are primarily concerned with research.

Traditional healer: A person who provides medical treatment and advice based on the traditional healing practices of his or her culture. May include shamans, diviners, acupuncturists, and herbalists. Traditional healers use plants and natural remedies instead of synthetic medications.

Illustrations and Photograph Credits

Photograph of Chromatography of Chlorophyll. Courtesy of Wikimedia Commons, Dominikmatus. 9 January 2013.

Photograph of St. John's Wort (*Hypericum perforatum*) Flowers. Courtesy of Wikimedia Commons, R.A. Nonenmacher. 3 July 2012.

Scientific Illustration of St. John's Wort (*Hypericum perforatum*) Plant Parts. Courtesy of Wikimedia Commons,Chris.urs-o. 4 October 2011. Originally from *Deutschlands Flora in Abbildunge*, Figure 60.

Sample Two-Peaked Chromatogram. Courtesy of Wikipedia, Klaas 1978. 3 September 2006.

Bibliographic Credits

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CHROMATOGRAPHY LAB BACKGROUND READING
STUDENT HANDOUT

Name:____ Date:_______________ Period:________

Depression Hurts

Have you seen TV commercials advertising medications to treat **depression**? The commercials show people who are unable to participate in daily activities. A dog waits by the front door, wanting to be walked. Breakfast sits on the table, uneaten. Someone lies in bed, twisted in the sheets, unwilling to get up in the morning. Hopeless. Lonely. Sad. Depression hurts, and antidepressant drugs may be able to help.

Depression is just one of the many mental and neurological disorders that affect people. About 1 in 10 adults in the U.S. report that they suffer from depression (CDC.gov). About 20% of teens will experience depression before they reach adulthood (teendepression.org).

What is depression? People sometimes say they are depressed when they feel sad, blue, unhappy, or down-in-the-dumps—but depression is much more than this. Most people feel sad some of the time; feeling unhappy or even miserable at times is a normal response to life's challenges. But if feelings of deep sadness, helplessness, hopelessness, or worthlessness last for weeks and interfere with everyday life, this sadness might better be called depression. Depression is a neurological disorder where feelings of sadness keep you from functioning normally for a long period of time.

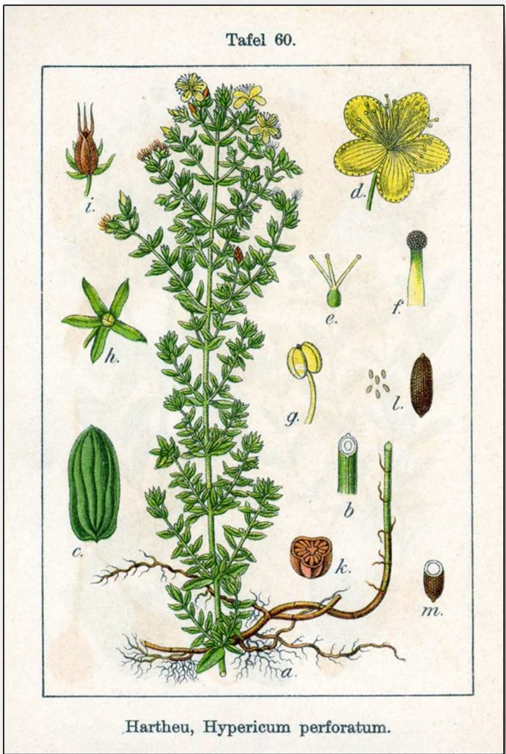
Depression is caused by an imbalance of certain **neurotransmitters** in the brain, including **serotonin**, dopamine, and norepinephrine. Serotonin helps regulates mood, appetite, and sleep. Low levels of serotonin are associated with depression.

Antidepressant drugs can help people suffering from depression by treating neurotransmitter imbalances. Some plants with **neuroactive chemicals** also may have the ability to help people suffering from depression.

An Uplifting Plant with a Strange Name

Plant extracts may contain chemicals which can treat depression and other neurological illnesses. St. John's wort is a common plant with pale yellow flowers—and a weird name! St. John's wort has a reputation for being anti-bacterial, anti-viral, and anti-inflammatory—that is quite a flower! St. John's wort has been used since ancient times to treat "nervous disorders." In recent years, scientists and doctors have studied St. John's wort as a treatment for depression.

Interestingly, many research studies suggest that St. John's wort is effective in treating mild to moderate depression and it has fewer side effects than prescription antidepressant drugs. Researchers don't totally understand the way that St. John's wort works. Some scientists think that a **bioactive chemical** in St. John's wort acts the same way that prescription anti-depressants work—by making more of the neurotransmitter serotonin available in the brain.



The leaves and flowers of St. John's wort contain many bioactive chemicals, including hypericin, pseudohypericin, hyperforin, and flavonoids. Researchers originally thought that hypericin was the neuroactive chemical in St. John's wort, but they now believe that other chemicals in St. John's wort may be responsible, or that several chemicals work together to improve depression.

Scientists have isolated many different chemicals from St. John's wort and are studying these chemicals individually and in combination to try to understand what components of the plant are responsible for its various healing properties. Other scientists continue research on products made from the leaves and flowers: capsules, tinctures, decoctions, infusions, and essential oils.

Chromatography: Combinations of Complex Components

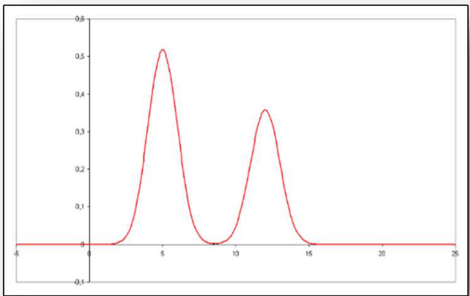
The plant world is filled with species that contain chemicals with medical properties, including neuroactive properties. Traditional healers use plants such as St. John's wort to treat illness, including mental illness. Current research seeks to understand the potential health benefits of different plants and is exploring whether documented health benefits are due to single chemicals found within the plant or several chemicals working together.

Chromatography is a laboratory technique used to separate the components of a complex mixture. This is a great first step in understanding how a medicinal plant works. After you separate the components, you can isolate the different chemicals found in the plant and determine: A) what those chemicals are; and B) what bioactive and neuroactive effects they have.

Scientists studying St. John's wort have isolated all the different compounds found in this plant but are still working to understand if the neuroactive effects are due to a single one of these chemicals or multiple chemicals working together.

Chromatography is a technique that is used in laboratories when the different components within a solution of gas or liquid need to be identified. There are many different techniques for conducting chromatography, but all require that a liquid or gas moves through a material; the different chemicals within the solution move through the material at different speeds, causing them to separate out.

In scientific labs, researchers use special chromatograph machines to separate the components within a solution of gas or liquid. These machines produce a **chromatograph**, a graph which shows peaks and patterns for the different chemicals in the solution.



In the lab portion of this lesson, you will use paper chromatography to determine whether a plant extract separates into different compounds that you can see. One reason why chromatography works is because different chemical compounds in the extract are different sizes. When these molecules dissolve in the **solvent** (water, acetone, or ethanol) and travel up the filter paper, small molecules travel faster and large molecules travel slower because they literally bump into the weave of the filter paper more often.

Chromatography Lab: Vocabulary List

Bioactive chemical: A chemical that interacts with or affect cells or tissues in animals.

Chromatography: A laboratory technique used to separate the components of a complex mixture.

Chromatogram: The paper strip on which the components of the mixture have been separated.

Chromatograph: A graph produced by a chromatograph machine which shows peaks and patterns for the different chemicals in the solution.

Filter paper: A porous type of paper for filtering liquids or separating liquids in paper chromatography.

Mortar and pestle: Together, a mortar and pestle are used to crush or grind up solid substances. The **mortar** is the cup in which ingredients are ground or crushed. The **pestle** is the heavy bat-like tool with a rounded bottom that grinds or crushes the ingredients in the mortar.

Neuroactive chemical: A chemical that interacts with or affects the brain or other nervous system cells in animals.

Neurotransmitter: Chemical that transmits information across the synapse to communicate from one neuron to another.

Plant extract: A liquid that contains plant chemicals. Types of plant extracts include infusions, decoctions, juices, essential oils, and tinctures.

Serotonin: A neurotransmitter that regulates mood, appetite, and sleep. Low levels of serotonin are associated with depression.

Solvent: A chemical that dissolves another chemical.

CHROMATOGRAPHY LAB PROCEDURE
STUDENT HANDOUT

Name:____ Date:_______________ Period:________

Your group will need the following laboratory materials:

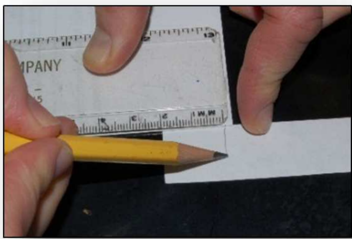
Item	Quantity Per Group
Goggles and gloves	1 per student
Spinach, purple cabbage and other vegetables, fruits, flowers or medicinal herbs	1
Plastic pipette	1
Filter paper strips	3
Scissors	1
Water, ethanol, or acetone	3 test tubes of the same solvent
Test tube rack	1
Pencil	1
Penny or other coin	1
Black Sharpie flip chart marker	1
Lab tape	1
Timer	1
Mortar and pestle	1
Metric ruler	1

Day One

1. Put on your gloves and safety goggles.

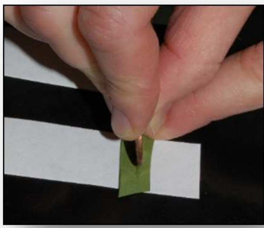
2. Prepare your three strips of filter paper:

    - Handle the paper by the edges, touching it as little as possible. The oils from your fingers can affect your experiment.
    - On the bottom end of each filter paper, use your ruler to measure 2 cm from the end of the paper and ***lightly*** draw a line across the paper with pencil, 2 cm from the bottom.
    - On the top end of two pieces of filter paper, write in pencil the name of the plant you are testing, such as "Ivy A" and "Ivy B". Write "Marker" on the top end of the third piece of filter paper.



3. Add plant material or marker to filter paper, using the procedures described below. Do all three of the procedures, one for each piece of filter paper.

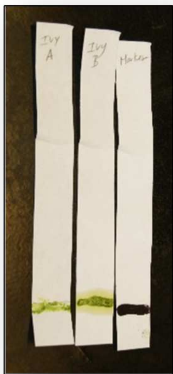
Filter Paper #1: Coin Rubbing Method



- Cut or tear the plant material into small strips. Lay a strip on the pencil line.
- Using a coin, rub the plant strip into the paper. The rubbing will transfer some plant material onto the filter paper. You can try both sides of the plant—one side may transfer to the paper more easily.
- When you are finished rubbing your coin on your plant, you should see a strong colored band on the filter paper. If you don't see this yet, rub another plant strip onto the paper.

Filter Paper #2: Mortar and Pestle Method

- Tear or cut the plant material into small pieces.
- Place these pieces in the mortar and grind them with the pestle until some liquid is produced. If no liquid forms, add a few drops of solvent—you can use water or ethanol—and then keep grinding with a mortar and pestle until the liquid is dark with the plant material.
- Using a plastic pipette, transfer some of the liquid out of the mortar and on to the filter paper—along the line that you drew on the paper.
- Wait for the liquid to dry completely. ***This is very important!***
- Pipette another two to three layers of liquid along the line—making sure that the liquid is totally dry before adding more.



Filter Paper #3: Marker Comparison

- Draw a thick band of ink with a Sharpie marker across the pencil line.

Day Two

1. Put on your gloves and safety goggles.

2. Start the experiment:

    - Ensure that you know which solvent is in your test tubes. Record this information in your lab notebook.
    - Place one filter paper strip into each test tube with the line of plant material towards the bottom.
    - Immediately record the start time in your notebook.

3. Stop the experiment:
    - When the solvent has moved nearly to the top of the filter paper, record the stop time in your notebook and remove the filter paper from the test tube.

4. Lay the filter paper down on a paper towel to allow the solvent to evaporate.

5. When the filter paper is totally dry, use a pencil to circle the visible bands.

6. These chromatograms fade quickly—by circling the bands now, you will have a record of where the different bands were at the end of the experiment.

7. Your teacher may want you to make a sketch of your chromatograms or take a photograph. Ask for these instructions.

8. Record the following data on the *Chromatography Data—Student Handout* or in your lab notebook:

    - Tape or glue the filter paper chromatograms into your notebook.
    - Next to each filter paper strip, record the following:
        a. The name of the plant you used.
        b. The method you used to get the plant extract onto the filter paper (mortar and pestle or coin rubbing).
        c. The solvent name.
        d. The amount of time the filter paper was in the solvent.

9. Follow your teacher's instructions on how to clean up your lab station and put away the lab equipment.

CHROMATOGRAPHY DATA
STUDENT HANDOUT

Name:____ Date:_______________ Period:________

Directions: Attach your three filter paper chromatograms onto this page. Next to each strip, record the following data:

a. The name of the plant you used.
b. The method you used to get the plant extract onto the filter paper (mortar and pestle or coin rubbing).
c. The solvent name.
d. The amount of time the filter paper was in the solvent.

CHROMATOGRAPHY RESULTS & CONCLUSIONS
STUDENT HANDOUT

Name:____ Date:_______________ Period:________

Results:

1. Explain why you see the different chemical compounds separate out as different color lines or smears on the filter paper.

2. How many bands can you see in your marker chromatography? Therefore, what does this tell you about the minimum number of chemical compounds that are in this ink? Why do you think there was a difference between the plant extract and the ink?

Conclusions:

3. Explain how chromatography is important in understanding the properties of medicinal plants.

4. After the class has had a chance to compare the results of the different groups, answer this question: Which solvent was most effective at separating compounds? Why do you think this might be?

CHROMATOGRAPHY VOCABULARY QUIZ
STUDENT HANDOUT

Name:____ Date:________________ Period:_________

Across

2. A machine-produced graph that shows peaks and patterns for the different chemicals in a solution.
5. A chemical that dissolves another chemical.
6. The cup in which ingredients are ground or crushed by the bat-like tool in #7.
7. A heavy bat like tool with a rounded bottom that grinds or crushes ingredients in the cup in #6.
8. A laboratory technique used to separate the components of a complex mixture.
11. The paper strip on which the components of the mixture have been separated.

Down

1. A chemical that interacts with or affects cells or tissues in animals (2 words).
3. A liquid that contains plant chemicals (2 words).
4. Chemical that sends information from one neuron to another.
5. A neurotransmitter that regulates mood, appetite, and sleep. Low levels of this are associated with depression.
9. A chemical that interacts with or affects the brain or other nervous system cells in animals.
10. A porous type of paper for filtering liquids or separating liquids in paper chromatography (2 words).