



SOP #: 1	TITLE: Methylene Chloride for liquid-liquid extraction
Author: Dexter Reasons Date of creation: 12/9/2023 Date of last review: 4/6/2025 Date of last revision: 4/6/2025	
Principal Investigator	Jane Doe
Location	Horizon 001
Designated Area	Horizon 001, south wall, fume hood
1. This standard operating procedure (SOP) is for	
The use of methylene chloride for liquid-liquid extraction.	
2. Chemicals, process, experiment, or equipment description	
A process designed to extract organic compounds from aqueous solutions. The aqueous solution and DCM are mixed, and a separatory funnel is used to separate the two layers. The process is appropriate for aqueous volumes of 30 mL to 60 mL. Each extraction requires 15 mL to 30 mL of DCM and takes about 20 minutes. Experiments should be done during normal working hours due to the health risks of DCM. This experiment only requires one person, but it is advised that an additional lab member (“buddy”) be present for safety.	
3. Hazards, risks, and controls	
We have identified the following hazards and risks and have implemented corresponding controls to enable the safe conduct of the proposed experiment or activity:	
Hazard and Risk	Controls (Engineering, Administrative, PPE)
1. Methylene chloride <u>a) Acute Exposure (Short-Term):</u> <u>Inhalation:</u> Breathing methylene chloride vapors can cause dizziness, headaches, nausea, and irritation of the respiratory tract. High concentrations may lead to confusion, unconsciousness, or even death due to central nervous system depression or oxygen displacement in the brain. <u>Skin Contact:</u> Prolonged exposure irritates the skin, potentially causing redness, burns, or dermatitis.	Substitution: We did not find any substitute chemical that has an equivalent function and effectiveness for this procedure. MeOH-DMC and i-PrOH-EtOAc did not provide sufficient separation of the phases, so the analyte was not sufficiently isolated. Engineering: a) Used in a fume hood to minimize inhalation exposure. Fume hoods are designed to capture vapors effectively, as methylene chloride is heavier than air and can accumulate in low-lying areas. b) Used in a closed loop system outside of a fume hood

<p>Eye Contact: Vapors or liquid can irritate the eyes, leading to redness, pain, or temporary vision impairment.</p> <p>Ingestion: Swallowing is rare but extremely dangerous, causing severe gastrointestinal distress and systemic toxicity.</p> <p>b) Chronic Exposure (Long-Term):</p> <p>Carcinogenicity: Methylene chloride is classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC). Studies link prolonged exposure to liver and lung cancers in animals, with potential risks to humans.</p> <p>Liver and Kidney Damage: Repeated exposure can harm these organs, as the body metabolizes methylene chloride into toxic compounds like carbon monoxide.</p> <p>Cardiovascular Effects: It can increase carboxyhemoglobin levels in the blood (due to metabolism into carbon monoxide), reducing oxygen delivery and posing risks to people with heart conditions.</p> <p>Neurotoxicity: Chronic inhalation may impair cognitive function, memory, and coordination, mimicking symptoms of carbon monoxide poisoning.</p> <p>c) Reactivity</p> <p>Methylene chloride reacts with strong oxidizers, alkalis, and certain metals (like aluminum), potentially releasing toxic gases like chlorine or phosgene.</p>	<p>Administrative & Work Practice:</p> <ol style="list-style-type: none"> Workers are trained on the hazards of methylene chloride, safe handling procedures, and emergency response protocols. Workers can recognize symptoms of overexposure (e.g., dizziness, headache, confusion). Workers handle methylene chloride during normal work hours and are with another worker as much as possible. Workers minimize exposure time and avoid unnecessary use. We use only the amount needed for the task, and clean up spills immediately with appropriate absorbent materials. Containers and work areas are clearly labeled with hazard warnings ("Danger: Contains Methylene Chloride – Inhalation Hazard"). Eating or drinking is prohibited in the lab. <p>Personal Protective Equipment:</p> <p>The following PPE have been selected as appropriate protection for our specific use of methylene chloride. They are available and are used when handling methylene chloride in our laboratory:</p> <ol style="list-style-type: none"> methylene chloride resistant gloves <ul style="list-style-type: none"> Example (Ansell brand): laminate film barrier, polyethylene vinyl alcohol chemtek butyl, chemtek viton/butyl impermeable aprons over lab coat chemical splash goggles (face shield over splash goggles if working with volumes over 100 ml or involves pressurization of the liquid)
<p>2. Glass apparatus presents a breaking hazard that would result in sharp pieces create a cut risk.</p>	<p>Administrative and Work Practice:</p> <ol style="list-style-type: none"> Funnel is cushioned on the ring clamp with plastic tubing. Glassware is inspected for cracks prior to each experiment.

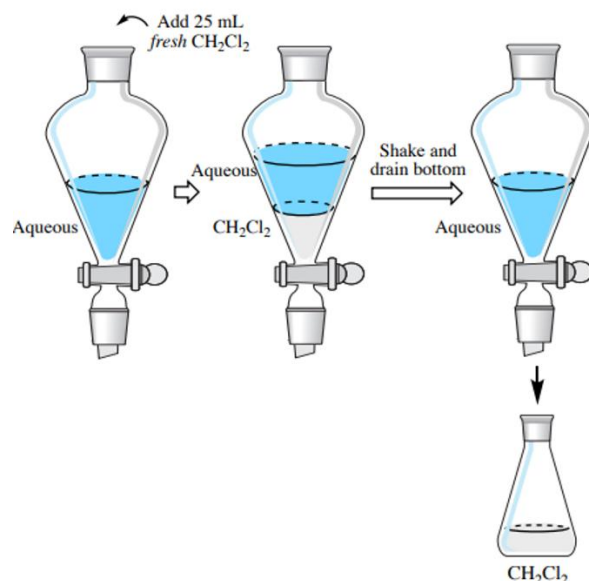
4. Step-by-step Methodology (Note precautions in blue)

Task or Activity	STEPS
Part I. Prepare the Setup	<ol style="list-style-type: none">1) Obtain a separatory funnel, and if the stopcock was removed for cleaning, replace it. Teflon stopcocks are used instead of glass stopcocks to prevent glass breaking. The stopcock should be set to a tightness that allows it to easily turn but not loose enough for liquid to seep around the joint.2) Set up the separatory funnel in a ring clamp attached to a ring stand or lattice work inside a fume hood. Use plastic tubing to cushion the funnel and ring.3) Check that the stopcock is in the “closed” position, and position an Erlenmeyer flask below the separatory funnel as a fail-safe.
Part II. Add the solutions	<ol style="list-style-type: none">1) Use a funnel to pour the aqueous solution into the separatory funnel.2) Use a funnel to pour an appropriate amount of DCM into the separatory funnel (use a volume of DCM that is approximately half of the volume of the aqueous solution.)
Part III. Mix the solutions	<ol style="list-style-type: none">1) Place the stopper on the funnel and hold the separatory funnel with one hand over the stopper and one hand at the base of the funnel. The funnel should remain inside the fume hood during this process.2) Gently invert the funnel and rock the fluid gently. Be sure to not mix too vigorously because DCM is prone to forming an emulsion. It’s possible that some liquid may seep around the stopper, so extra precaution should be taken to ensure that any spilled drops do not touch exposed skin.

- 3) While the funnel is inverted, "vent" the funnel by briefly opening the stopcock. This prevents any built up pressure from the solutions mixing.
- 4) Repeat the steps of rocking and venting for approximately one minute.

Part IV. Separate the layers

- 1) Place separatory funnel upright on the ring clamp and allow the layers to fully separate. DCM is more dense than water, so the organic layer will be the lower layer.
- 2) Remove the stopper from the separatory funnel. If the stopper is not removed, negative pressure will form while the liquid drains.
- 3) Drain the bottom layer into a clean Erlenmeyer flask, stopping when the interface just enters the stopcock. Be sure the tip of the separatory funnel is nestled in the Erlenmeyer flask to prevent splashing, particularly of DCM.
- 4) Keep the remaining aqueous layer in the separatory funnel or a separate Erlenmeyer flask until confirmation that the compound has been extracted.



Source: [https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Organic_Chemistry_Lab_Techniques_\(Nichols\)/04%3A_Extraction/4.06%3A_Step-by-Step_Procedures_For_Extractions](https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Organic_Chemistry_Lab_Techniques_(Nichols)/04%3A_Extraction/4.06%3A_Step-by-Step_Procedures_For_Extractions)

5. Clean-up, decontamination, and waste disposal

Dispose of all chemicals in appropriate hazardous waste containers. Rinse all glassware with acetone to remove organic material, and dispose of the first wash of acetone in the same hazardous waste container as DCM. When disposing of DCM, keep the hazardous waste container in the same fume hood as the experiment so the DCM fumes are contained within the fume hood.

6. Special handling procedures, transport, and storage requirements

Methylene chloride is stored in tightly sealed, labeled containers in the flammable cabinet under hood 987 in Horizon 001. The storage area is cool, well-ventilated and away from heat sources, open flames, or incompatible materials (e.g., strong oxidizers, aluminum).

7. Emergency equipment and procedures

Item	Location
Eyewash / safety shower	Safety shower in the hallway outside of Horizon 001 and eyewash beside the sink.
First aid kit	Top shelf of the book shelf next to the entrance of 001.
Chemical spill kit	Second shelf of the book shelf next to the entrance of 001.
Fire extinguisher	Under sink in 001.
Fire alarm manual pull station	Hallway directly outside of 001.
Telephone	Desk in the adjoining office.

Emergency	What to Do
Methylene chloride splashes on the eyes	<ol style="list-style-type: none"> 1. As needed, call 911 to seek emergency medical help. 2. Assist exposed person away from the incident or source of exposure to the eyewash Do this only if able and personal safety is not compromised. 3. Push the eyewash lever to start the water flowing. 4. Hold your eyes open, move your eyes up and down and side to side to get the water under your eyelids. 5. Stay under the water for at least 15 minutes to wash off the chemicals. 6. Report incident to Jane Doe (000-000-0000). 7. Seek follow-up medical treatment at the nearest ophthalmologist during normal business hours or the nearest emergency room after business hours.
Methylene chloride splashes on the skin	<ol style="list-style-type: none"> 1. As needed, call 911 to seek emergency medical help. 2. Assist exposed person away from the incident or source of exposure to the emergency shower. Do this only if able and personal safety is not compromised. 3. Pull the safety shower lever to start the water flow.

	<ol style="list-style-type: none"> 4. Remove all contaminated clothing and shoes to effectively wash chemicals off your skin. 5. Stay under the water for at least 15 minutes to wash off the chemicals. 6. Report incident to Jane Doe (000-000-0000).. 7. Seek follow-up medical treatment at the nearest clinic during normal business hours or the nearest emergency room after business hours.
If methylene chloride is inhaled	<ol style="list-style-type: none"> 1. Call 911 to seek emergency medical help. 2. Assist exposed person away laboratory and into an area with fresh air. 3. Wait for emergency responder to arrive and take the person to the nearest emergency room.
If methylene chloride is spilled	<p>If spill occur inside a fume hood and it is well-contained, clean up the spill using your spill kit. Apply the solvent absorbent to absorb the liquid. Collect contaminated absorbents in a sealable plastic bag, double bag it, then place them in a solid waste container. Scrub the spill area with soap and water. Collect all clean-up materials as hazardous solid waste and request a hazardous waste pickup immediately.</p> <p>If spill occur outside of a fume hood:</p> <ol style="list-style-type: none"> 1. Assist anyone who may have been contaminated or injured during the spill. 2. Post “DO NOT ENTER” on entrance door and evacuate the area. 3. Call EH&S at (803) 777-5269, (803) 422-2122, (803) 351-9874 4. Do not re-enter until Emergency Responders have cleaned up the spill and declare the area safe for reentry.
Life-threatening emergencies	<ol style="list-style-type: none"> 1. Call 911. 2. Provide dispatch the following information: your name and call back number, location of incident, material released, if known, or if there are any injured person and their location. 3. If building needs to be evacuated, pull the nearest fire alarm. 4. Exit the building using the nearest stairway. 5. Proceed to designated assembly area. 6. Provide information to emergency responders as able.
Facility malfunction (i. e., power outages,	Call (803) 777-9675 to report and request work order.

plumbing leaks, fume hood)	
If needing medical care due to injury at work (personnel compensated by USC only)	See Section 10 Attachments for procedures on how to obtain medical treatment for work-related injuries of USC employees. After seeking medical care, any injury or illness involving any of the following: <u>death, amputation, loss of an eye</u> , is expected to or results in a hospitalization, must be reported to EH&S at (803) 777-5269 and Buddy Harley at (803) 528-8191 within 24 hr.
Incident Reporting	Report all incidents to your Supervisor or Principal Investigator, then to EH&S by completing the incident report form once emergency response has been completed (i.e., Emergency Responders have declared the incident area "All Clear", spill has been cleaned up, and necessary medical care has been initiated).

8. Training requirements

- Chemical and Lab Safety Training (EH&S)
- Review of Methylene Chloride safety data sheet (Lab PI)
- Demonstrate proficiency in step-by step methodology including safety procedures (Lab PI)
- Demonstrate proficiency in executing emergency response procedures (Lab PI)
- Hazardous Waste Training (EH&S)

9. Review, Approval and Authorized Users

Approved by Principal Investigator: Jane Doe, *Jane Doe*, 12/9/2023

Prepared by Author: Dexter Reasons, *Dexter Reasons*, 12/9/2023

Reviewed by EH&S Chemical Hygiene Officer: Jocelyn Locke, *Jocelyn Locke*, 12/15/2023

Authorized Users

I, an Authorized User of this Standard Operating Procedure for the Use of Methylene Chloride for liquid-liquid extractions, agree by signing below, that I have completed all required trainings listed in Section 8. I have read and understand the content of this SOP, and will follow all aspects of this SOP, including but not limited to the proper use of PPE, safe handling of hazardous materials, precautionary measures, methodology, emergency procedures, and other instructions. I agree that I have received lab-specific training on the safe use of hazardous material, equipment, and processes described in this SOP.

