Standard Operating Procedure

Task: Liquid Dispensing
Date: 09/04/2018
Revision Date:

Background

- The addition of liquid reagents, solvents, or solution is a fundamental skill in synthetic chemistry.
- There are several methods by which a liquid can be added to a reaction mixture.
- This SOP briefly introduces different liquid dispensing methods and discusses the ideal usage scenarios.

Training Requirements:

- Lab safety training

Potential Hazards:

- Hazards associated with specific chemicals being handled
- Needle sticks

Special PPE Requirements:

- Choose PPE based on specific chemical hazards

Procedures:

A. Pipet (Pasteur pipette)

Features.

- Non-quantitative
- Typically used in the volume range 0.5–30 mL
- Typical pipet bulb dispenses ~1 mL, with a single drop delivering ~0.01 mL
- Air-free use only inside of a glovebox

Summary of protocol.

- Attach bulb to pipet.
- Depress and hold bulb, insert into liquid, slowly release the bulb to draw up liquid.
- Depress bulb to dispense liquid.

Example use cases.

- Solvent addition in small scale reactions run in 20 mL scintillation vials
- Solvent addition for washing or extracting solids
- Weighing liquid reagent by addition to tared vial

B. Automatic pipet

Features.

- Quantitative addition
- Automated addition possible with some models
- Can be used in the volume range 0.001–10 mL
- Most automatic pipets have poor compatibility with organic solvents (backpressure pipets are a good alternative, see below)
- Air-free use only inside of a glovebox

Summary of protocol.

- Set desired volume to be dispensed on pipet
- Attach plastic tip appropriate for the volume and chemically compatible with the liquid
- Depress plunger until first resistance point is felt (do not press too hard), insert into liquid, and slowly release to draw up liquid. Watch for drips when working with dense or low viscosity liquids.
- Depress plunger fully, passing the first resistance point, to dispense the liquid.

Example use cases.

- Precise addition of water or aqueous solutions
- pH titrations

C. Syringe

Features.

- Quantitative addition
- Typically used in the volume range 0.001–10 mL
- Glass or plastic casing construction available
- Plastic casings are not always compatible with organic solvents or reagents
- Not suitable for large volumes
- Not suitable for pyrophoric reagents, except in small volumes
- Air-free use on Schlenk/vacuum line or inside of a glovebox

Summary of protocol.

- Insert needle tip into liquid, draw back plunger slowly, slightly past desired volume
- Ensure that no bubbles are present
- Discard excess liquid by depressing plunger until the desired volume is present
- Dispense liquids by depressing plunger
- Note that there is a “dead volume” in the syringe needle, which should be filled with a small amount of liquid sample after dispensing.

Example use cases.

- Addition of liquid reagent to a reaction
- Addition of liquid internal standard to a reaction
- Removal of known volume aliquots for reaction analysis

D. Electronic repeating positive displacement pipette (automatic syringe)

Features.

- Quantitative addition
- Automated addition possible with some models
- Typically used in the volume range 0.001–10 mL
- Good organic solvent compatibility: tips are made from robust plastic
- The plunger-type action makes this pipette particularly well suited for organic solvents of varying densities and a variety of vapor pressures.
- Adjustable aspiration and dispensing speeds combined with relatively wide tip openings allow handling of viscous organic or aqueous solutions that cannot typically be aspirated with glass μ-syringes due to their generally thin needles.
- Air-free use only inside of a glovebox

Summary of protocol.

- Select the dispensing mode (shown for Eppendorf Repeater E3/E3X)

Mode	Description	E3	E3x
Pip	Dispensing in one dispensing step.	■	■
Dis	Dispensing in identical dispensing steps.	■	■
Ads	Automatic dispensing in identical dispensing steps.	■	■
Seq	Dispensing of different dispensing steps.	–	■
Asp	Aspiration of identical volumes.	–	■
A/D	Aspiration of an unknown amount of liquid. Dispensing in identical dispensing steps.	–	■
Ttr	Dispensing of a liquid with the actuate key pressed down.	–	■
Opt	Adjustment of device settings (language, volume, etc.).	■	■

  - **Pip**ette mode allows for normal pipetting workflow (i.e. single volume aspirate and dispense)
  - **Dis**pense mode is useful for dispensing identical volumes of solvents, reagents, or stock solutions for reaction screening, high-throughput catalysis, titrations, or parallel NMR experiments
  - **Seq**uential mode allows you to program and save a set of varying volumes (increasing, decreasing, alternating etc.) for dispensing and will prompt you when necessary to aspirate more stock solution to continue with your series. Useful for titrations and concentration dependence studies of parallel reactions (substrate, catalyst loading, additives, etc.). **NOTE: These series can be saved as favorites, so you will not have to reprogram the pipette for every experiment**

- Set desired volume(s).
- Set aspiration speed and dispensing speed.
- Set number of dispensing steps.
- Aspirate the liquid.
- Press the actuation key to take up the liquid
- Hold the dispensing tip over the first well in the destination vessel and press the actuation key to dispense the liquid.

Notes

- Requires a bit of excess stock solution as the tips are calibrated to dispense with a small buffer of your solution to prevent imprecise dispensing or dispensing of air or solvent vapor. Amount of excess required is directly related to max tip volume
- Tips cost $1 each
- Link to manuals: https://online-shop.eppendorf.us/US-en/Manual-Liquid-Handling-44563/Pipettes-44564/RepeaterE3-E3x-PF-135444.html

Example use cases.

- Known volume addition of organic liquid reagent to a reaction
- Known volume addition of organic liquid internal standard to a reaction
- Removal of known volume aliquots for reaction analysis
- Organic solvent addition to multiple parallel reactions
- Automated solvent or reagent additions with programmed variations

E. Cannula

Features.

- Non-quantitative
- Typically used in the volume range 3–1000 mL
- Air-free technique for Schlenk/vacuum line

Summary of protocol.

- Pierce cannula through septum in “sending” flask open to inert gas bubbler. A stream of the inert gas should be directed through the cannula.
- Pierce cannula through septum of “receiving” flask open to inert gas bubbler. Keep cannula above any liquid in either flask.
- Close connection to bubbler on receiving flask.
- Insert a vent needle in septum of receiving flask.
- Slowly insert cannula into liquid of sending flask.
- Dispense desired amount of liquid.
- Remove cannula from liquid of sending flask.
- Remove vent needle.
- Open receiving flask to bubbler.
- Remove cannula carefully from both flasks.

Example use cases.

- Addition of estimated volume of solvent to an air-free reaction
- Addition of solvent to for washing/extraction under air-free conditions
- Addition of reagent or solution to addition funnel for quantitative additions under air-free conditions (see below)

F. Addition funnel

Features.

- Quantitative addition
- Typically used in the volume range 5–500 mL
- Air-free technique for Schlenk/vacuum line

Summary of protocol.

- Test stopcocks and funnel the night before to minimize the chance of leaks during addition.
- Attach addition funnel to “receiving” vessel to which liquid will be added. Place under inert atmosphere using standard Schlenk techniques, if needed.
- Ensure that addition valve is closed.
- Add liquid to addition funnel, using graduation marks for quantitation of liquid volume.
- Some addition funnels are equipped with a pressure equilibration arm; ensure that this valve is open during addition to preserve inert atmosphere of the addition funnel and allow consistent addition of the reagent. If the addition funnel does not have a pressure equilibration arm, a pressure-equalizing (inert) gas inlet must be provided.
- Slowly open addition valve to allow liquid to dispense into receiving flask. Rate of addition can be controlled based on how far the valve is opened.

Example use cases.

- Quantitative addition of solvent or reagent under air-free conditions
- Controlled quantitative addition of pyrophoric reagents for large-scale reactions
- Titrations where total volume added must be known

G. Volumetric flasks and graduated cylinders

Features.

- Quantitative addition
- Typically used in the volume range 0.5–1000 mL
- Air-free only inside of a glovebox

Summary of protocol.

- Add liquid to container. Volumetric flasks are only accurate for the specific volume of the flask; fill to the line to achieve the stated volume. Graduated cylinders can measure a range of volumes; fill to the desired volume in the graduation range of the container.
- Pour liquids into “receiving” flask to dispense.

Example use cases.

- Quantitative solvent addition
- Quantitative reagent addition for large scale reactions
- Dilutions for analysis

H. Vacuum transfer

Features.

- Non-quantitative
- Typically used in the volume range 1–100 mL
- Air-free technique used with vacuum lines

Summary of protocol.

- Set up vacuum line and make sure that it is pumping down properly and that there are no leaks.
- Assemble your vac transfer apparatus.
- Degas the contents of the sending flask, usually by the freeze-pump-thaw method.
- Place the apparatus under static vacuum. Open the valve to the receiving flask.
- Apply a dry ice / acetone cooling bath to the receiving flask. Liquid nitrogen is not recommended because you are cooling a closed system under static vacuum.
- Slowly open the valve on the sending flask.
- Note: when transferring solvents off of desiccants, do not transfer all of the solvent. This is especially true of ethers or other peroxide-forming solvents: any peroxides will concentrate as the transfer continues, and could detonate if taken to dryness.
- Once you have transferred the desired amount of solvent, close the sending flask first. Allow a few minutes for any remaining vapors to transfer to the receiving flask. Then close the receiving flask and pump down the system. Depending on your desired use, it may be useful to perform at least one freeze-pump-thaw cycle on the receiving flask (if no deflection is observed, there was no leak).

Example use cases.

- Addition of solvent to a reaction flask under vacuum
- Addition of deuterated solvent to J-Young NMR tube under vacuum
- Rarely used for liquid reagent addition

References and Related SOPs:

- Vacuum Traps
- Vacuum Transfer
- Degassing

Appendix 1

^{1}H NMR spectra of common organic solvents manipulated in Combitips Advanced® Positive Displacement Pipet Tips for Eppendorf® Repeaters. The top (red) spectra are the pure solvent manipulated using glass pipets and the bottom (blue) spectra are 600 μL aliquots that underwent five aspiration/dispensing cycles before being transferred to an NMR tube for analysis.

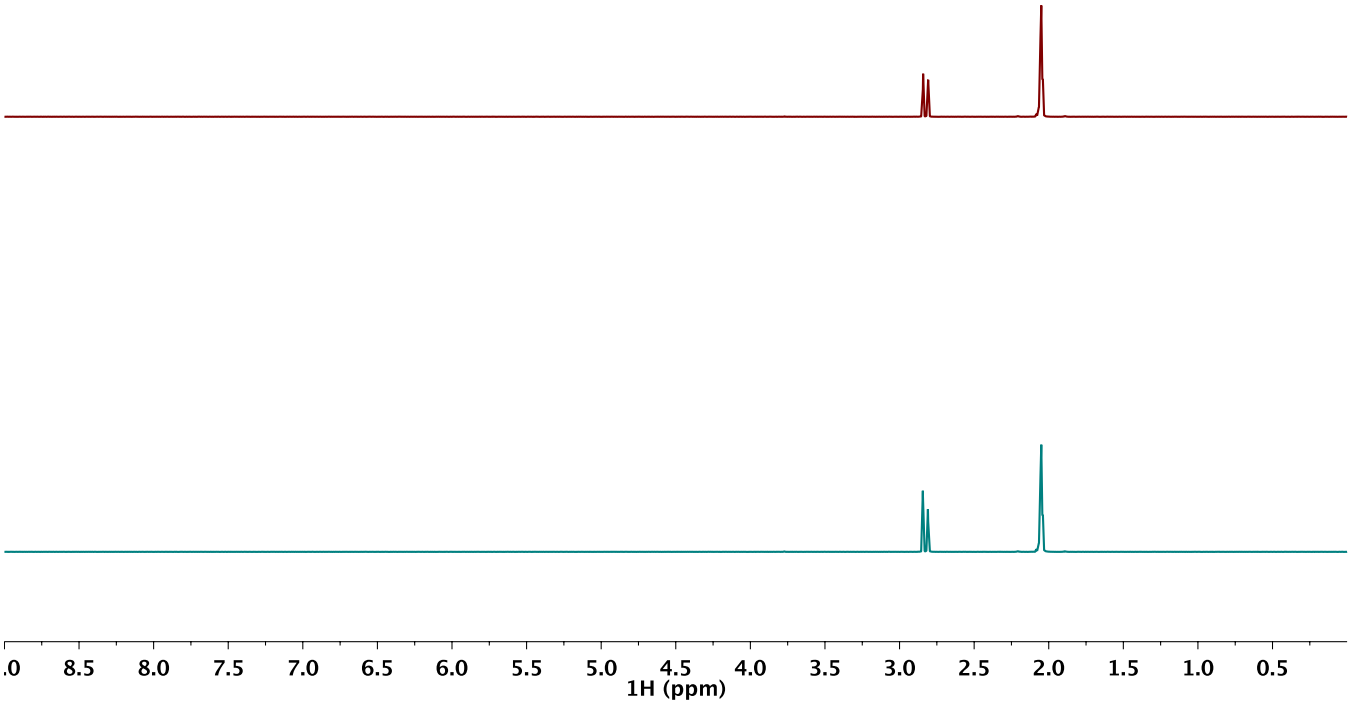

Figure 1. Acetone

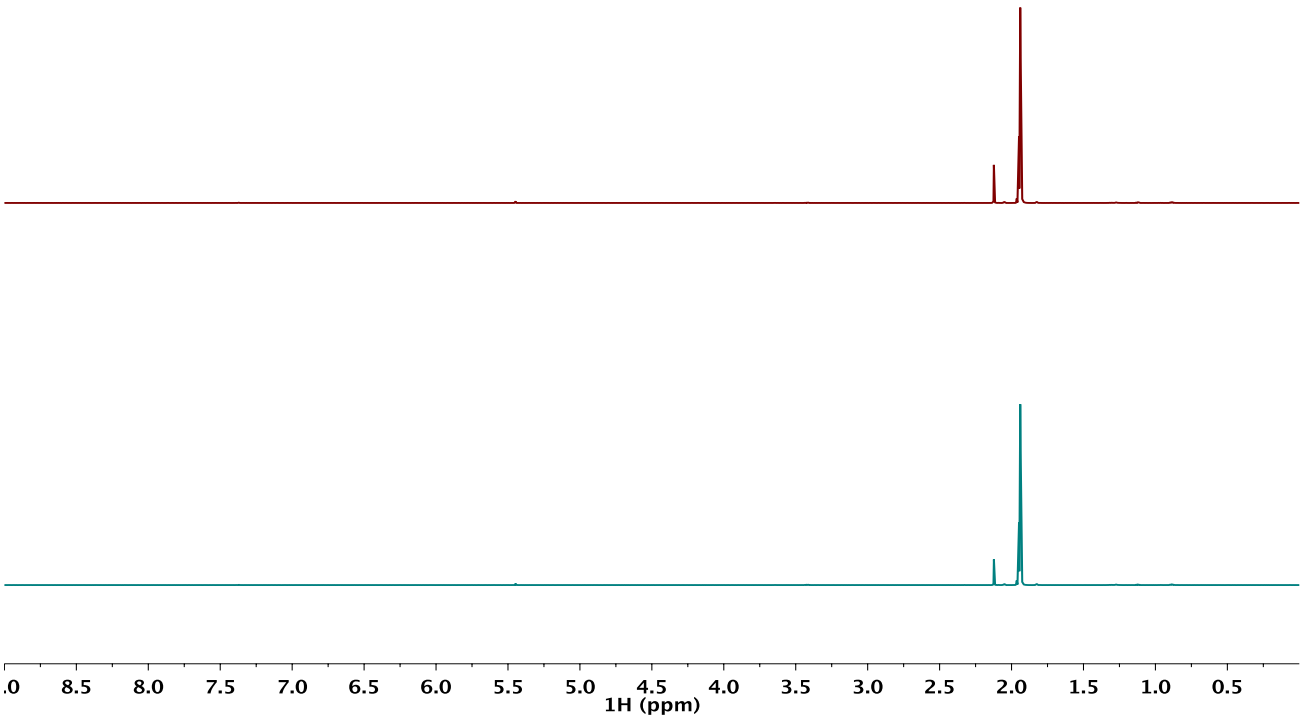

Figure 2. Acetonitrile

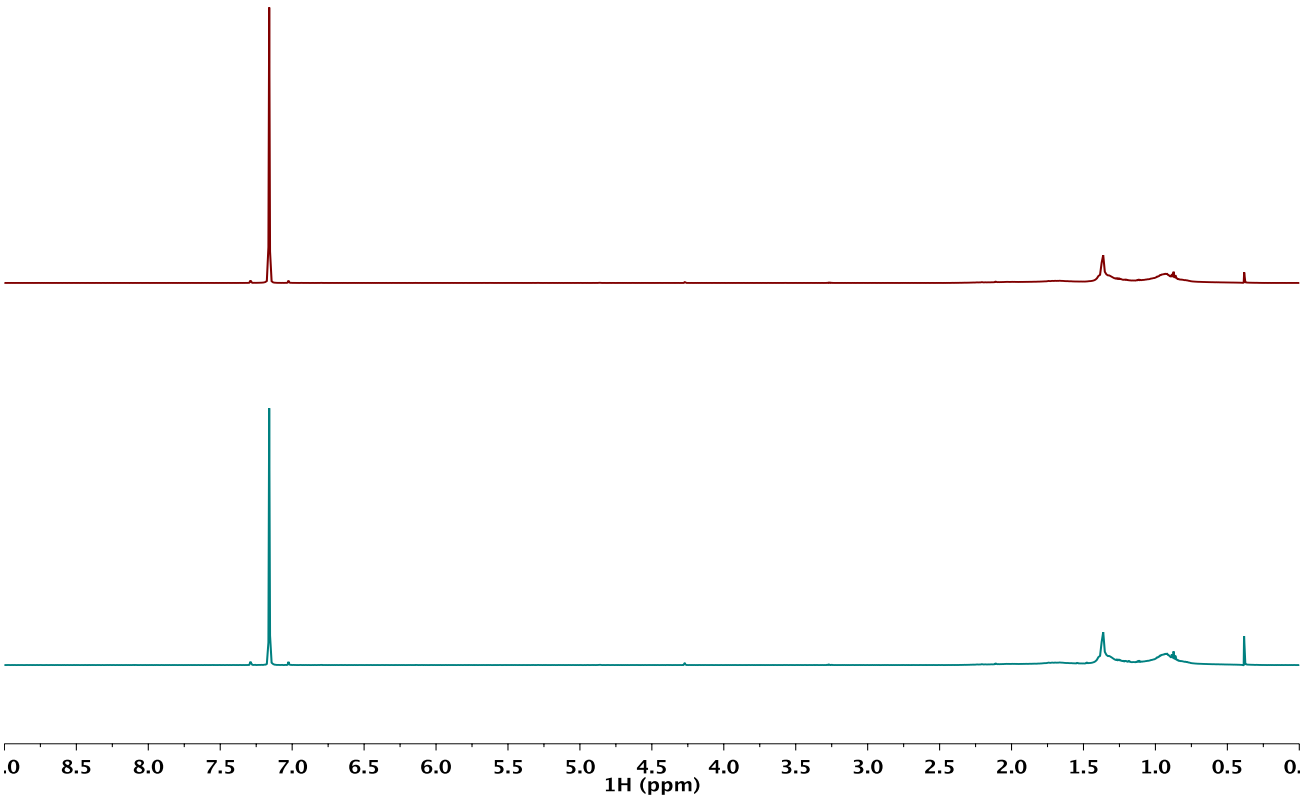

Figure 3. Benzene

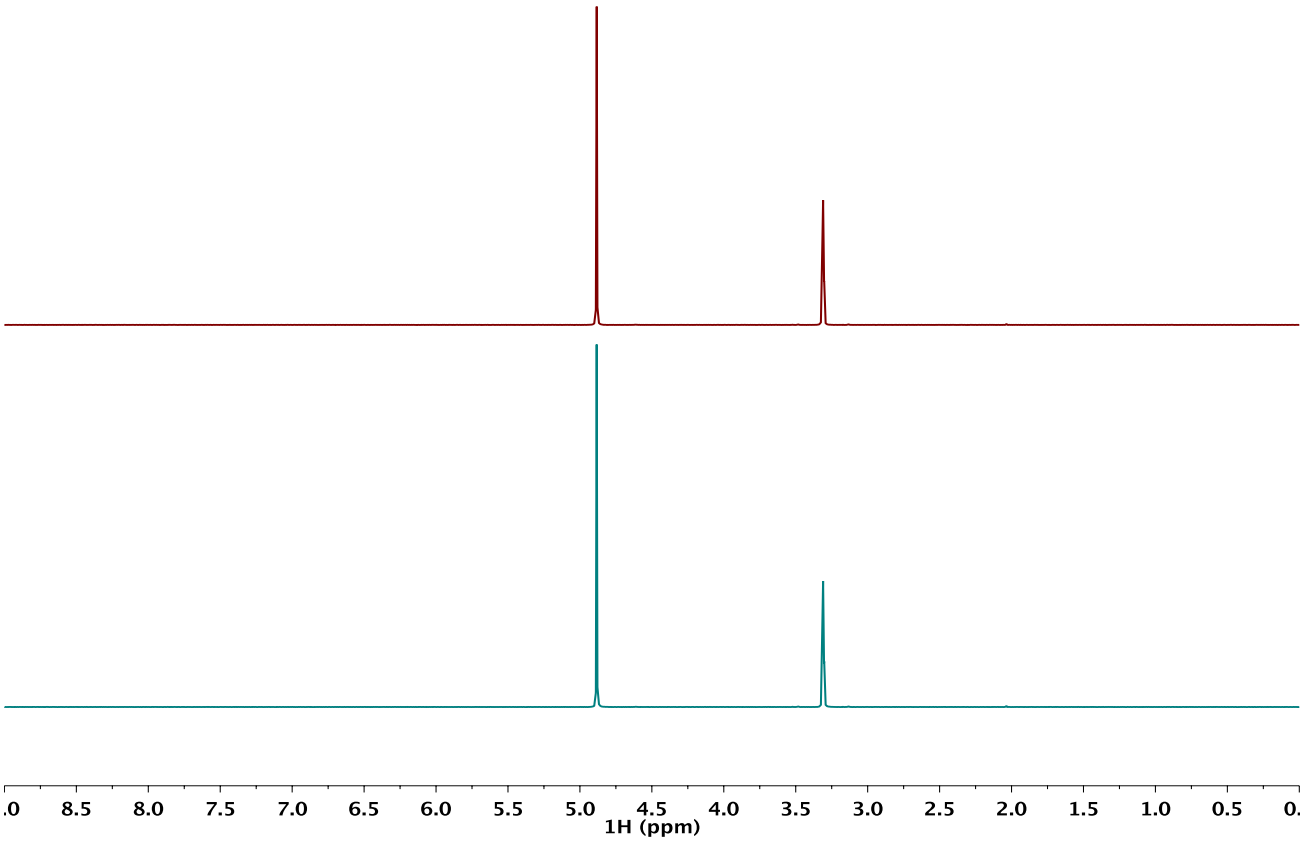

Figure 4. Methanol

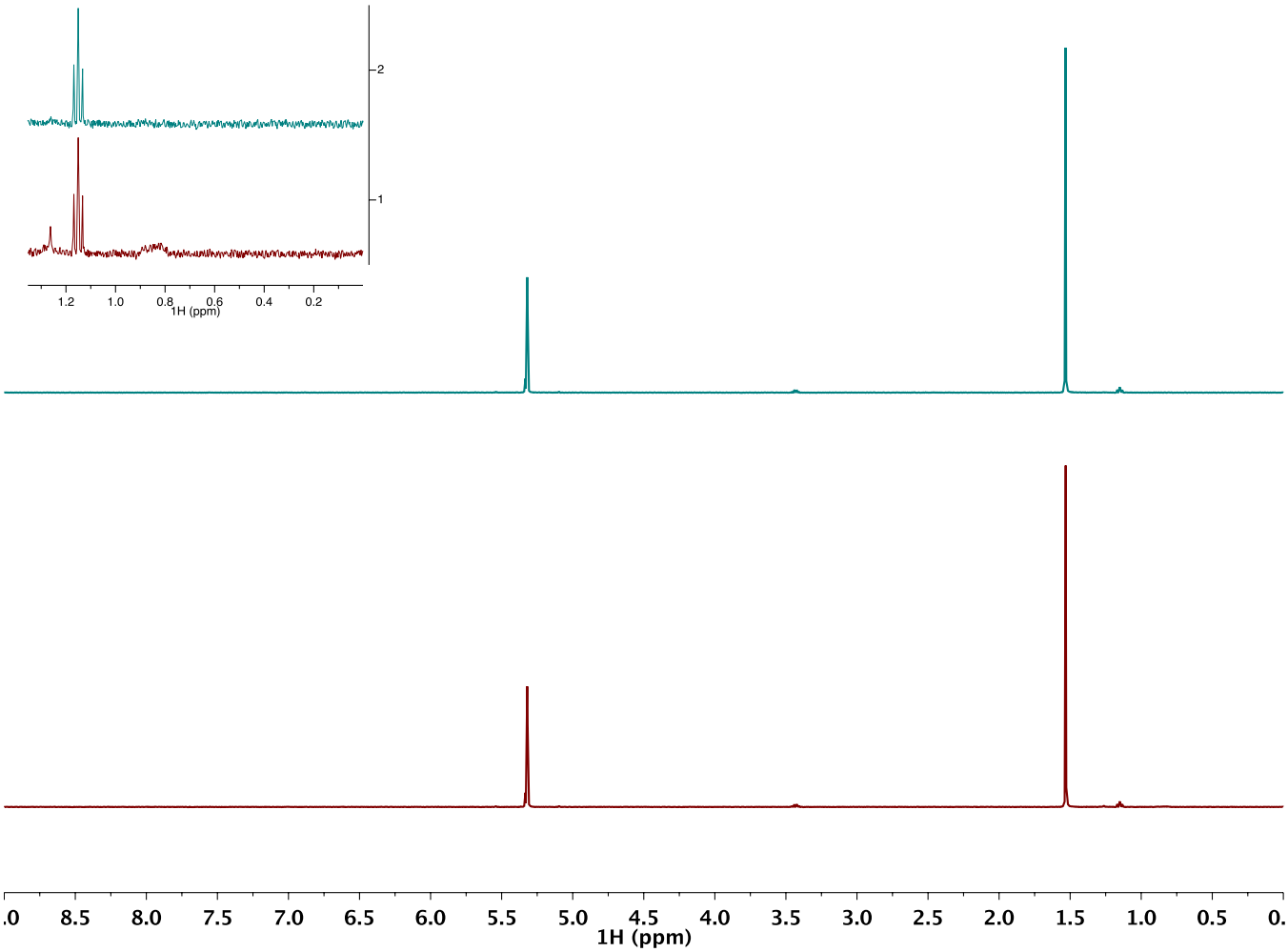

Figure 5. Dichloromethane

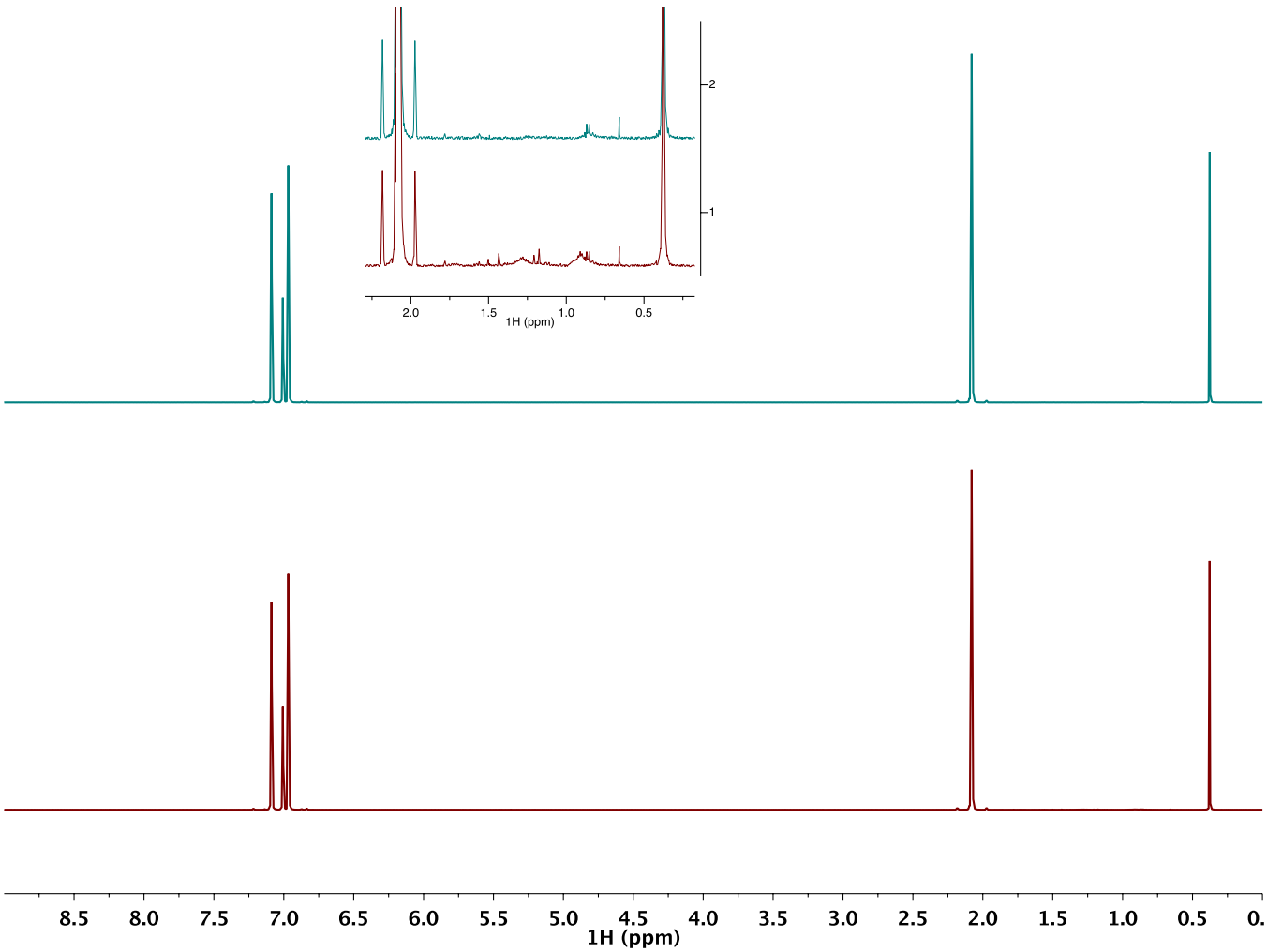

Figure 6. Benzene