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A Procedure for the Supercritical Fluid Extraction of Coal Samples, with Subsequent Analysis of Extracted Hydrocarbons

By Jonathan J. Kolak

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Key to Abbreviations

30D:	30% (v/v) dichloromethane in hexane
30M:	30% (v/v) methanol in dichloromethane
ASTM	American Society for Testing and Materials
CHP:	chemical hygiene plan
DCM:	dichloromethane
DDW:	distilled, deionized water
d-PAH:	deuterated polycyclic aromatic hydrocarbon
EOM:	extractable organic matter
GC:	gas chromatography
GC-MS:	gas chromatography-mass spectrometry
ISTD:	internal standard
JHA:	job hazard analysis
MeOH:	methanol
MPa:	megaPascal
NEVAP:	nitrogen evaporator; a device used for evaporating relatively small volumes of solvent under a gentle stream of nitrogen
NIST:	National Institute for Standards and Technology
NSO:	a nitrogen-, sulfur-, and(or) oxygen-containing organic compound
PAH:	polycyclic aromatic hydrocarbon
Ph:	phytane
Pr:	pristane
PPE:	personal protective equipment
psi:	pounds per square inch
PTFE:	polytetrafluoroethylene
RT:	retention time
SARA:	saturates, aromatics, resins, and asphaltenes
SFC:	supercritical fluid chromatography
SFE:	supercritical fluid extraction
SIM:	selective ion monitoring
soln:	solution
SRM:	standard reference material
SURR:	surrogate

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Introduction

This report provides a detailed, step-by-step procedure for conducting extractions with supercritical carbon dioxide (CO_2) using the ISCO SFX220 supercritical fluid extraction system. Protocols for the subsequent separation and analysis of extracted hydrocarbons (fig. 1) are also included in this report. These procedures were developed under the auspices of the project, "Assessment of geologic reservoirs for carbon dioxide sequestration" (see <http://pubs.usgs.gov/fs/fs026-03/fs026-03.pdf>), to investigate possible environmental ramifications associated with CO_2 storage (sequestration) in geologic reservoirs, such as deep (~1 km below land surface) coal beds.

Supercritical CO_2 has been used previously to extract contaminants from geologic matrices. Pressure-temperature conditions within deep coal beds may render CO_2 supercritical. In this context, the ability of supercritical CO_2 to extract contaminants from geologic materials may serve to mobilize noxious compounds from coal, possibly complicating storage efforts. There currently exists little information on the physicochemical interactions between supercritical CO_2 and coal in this setting. The procedures described herein were developed to improve the understanding of these interactions and provide insight into the fate of CO_2 and contaminants during simulated CO_2 injections.

Instrument Overview and Specifications

The instruments used in the extraction procedure are an ISCO SFX220 extraction system and two model 260D syringe pumps. This system is capable of conducting extractions at temperatures up to 150°C and pressures up to 7500 psi (~517 bar or 51.75 MPa). One syringe pump is dedicated for use with CO_2 and is connected to a cylinder of SFE-grade CO_2. The second pump, that is, the modifier pump, is used to supply additional fluids, thus modifying the properties of the CO_2 extracting fluid. The most commonly used modifiers include water, methanol, or hexane. The modifier is supplied to the pump through a connection to a glass reservoir (fig. 2).

It should be noted that these extractions are conducted at elevated pressures. Furthermore, ***both*** the interior of the extraction vessel ***and*** the space outside the vessel are pressurized with the same extraction fluid in order to maintain extraction vessel integrity. At the conclusion of an extraction, the external pressurizing fluid is vented as a fine mist outside the extraction unit. Therefore, ***when conducting extractions using a toxic and(or) flammable organic solvent as a modifier, a trap must be set up to collect this vented material, thus minimizing exposure and(or) reducing the risk of fires.***

Labware and Laboratory Glassware Preparation

Objective: To ensure that all labware, such as stainless steel spatulas and PTFE stopcocks, and all laboratory glassware contacting samples are free of contamination. [Note: Do NOT wash nor bake out the SFE vessels. Instead, simply rinse vessel components three times each with acetone and DCM, and allow to dry in a covered beaker under a fume hood.]

Reagents Needed:

Alconox or similar detergent
Deionized (DI) water
Deionized distilled water (DDW) or "organic-free" or Milli-Q (18.2 MΩ) water
Acetone (pesticide research grade or better) in PTFE squirt bottle
Dichloromethane (pesticide research grade or better) in PTFE squirt bottle
Aluminum foil
Laboratory muffle furnace

1. Wear gloves, such as vinyl or nitrile, when handling glassware to minimize contamination.

2. Wash items in a mixture of detergent and hot water. (For disposable glass Pasteur pipettes, skip the detergent wash and proceed directly to step 3.)

3. Rinse items three times each with tap water, followed by DI water, and then by DDW.

4. Let items drain ~ 5-10 min, then place glassware on a dry Pyrex baking dish and dry in an oven at 60°C.

5. After items have dried completely, inspect items to ensure that they are free of detergent residue. (Repeat steps 2-4 for each item containing detergent residue.)

For labware:

6. Remove dried PTFE items and store in ashed glass jar, sealed with aluminum foil. Remove dried stainless steel spatulas from oven, rinse three times each with acetone and DCM, allow to dry under a fume hood, and store in a clean, covered container.

For laboratory glassware:

7. Remove glassware from the oven. Using aluminum foil, loosely cover the openings of the dried, residue-free glassware.

8. Place the dried, foil-covered glassware in a muffle furnace, and bake out, that is, ash, the glassware at 450°C for 6-8 h to remove any remaining organic contaminants.

9. After the glassware has been ashed and then cooled, seal the aluminum foil and store the glassware until needed.

Sample Preparation

1. Coal samples are collected in the field and immediately stored double-bagged in plastic to minimize oxidation and water loss.

Representative channel samples or samples from drill core section are first sent to an accredited contract lab for grinding, sieving (-60 mesh), and proximate/ultimate analyses.

Channel bench samples are also collected. Coal core plugs are taken from the channel bench samples at the USGS using a drill press fitted with a diamond core bit and a water swivel (figs. 3A-D). After coring, the remainder of the channel bench sample is sent to an accredited contract laboratory for grinding, sieving (-60 mesh), and proximate/ultimate analysis.

For coal core plugs:

2. Coal core plug samples are immediately transferred into the SFE vessel. The diamond core bit and SFE vessel have nearly identical inner diameters, hence, a piston is used to drive the core plug from the core bit into the SFE vessel. The extraction vessel is then fully assembled (see section under "Instrument Set Up") and the extraction is begun immediately afterwards.

For ground coal samples:

3. The ground coal samples are shipped from the laboratory back to the USGS, and then the samples are transferred to an ashed Pyrex drying tray. The ground coal samples are dried for approximately 24 h under air in a gravity-convection oven at 40°C until no more moisture is evolved.

4. The dried samples are then stored in ashed glass jars with PTFE-lined lids.

5. Immediately prior to extraction, a split is taken from the ground coal sample using a Jones-type riffle splitter. A 1.000-g sample is typically used.

6. The split sample is then transferred to a beaker and placed in a gravity convection oven at 40°C for several hours to remove absorbed moisture. The ground coal sample is now ready for extraction.

Reagents Needed for Supercritical Fluid Extraction of Prepared Coal Samples

CO_2: Cylinder of "SFE" or "SFC"-grade CO_2. The cylinder must have an edductor (dip) tube so that liquid (not gaseous) CO_2 is drawn from the cylinder. NOTE: The CO_2 cylinder should NOT be pressurized with a He headspace. With time, the He will dissolve into the liquid CO_2, and the fluid withdrawn from the cylinder may contain up to several percent He.

DCM: High-purity (pesticide grade or better) – used to rinse extraction vessel and fritted filter units in between samples.

Baked glass wool and/or baked quartz sand: Ashed in muffle furnace for 6 h at 450°C to drive off remnant organics; used in packing extraction vessels and as method blanks.

Hexane: High-purity (pesticide grade or better); used in the solvent trap to capture extracted hydrocarbons.

Ice: For ice-water bath used to chill the solvent trap.

Water: If using water as a solvent modifier, use "organic-free" or Milli-Q (18.2 MΩ) water.

Instrument Set Up

Preparing the Instrument:

1. Prepare the solvent traps in advance so that the solvent can be chilled prior to extraction. Pour approximately 100 ml of hexane into a 125-ml capacity glass flask, cap, and store at 4°C until needed in the extraction. (Hexane is used in the solvent traps to precipitate any asphaltenes that may be extracted.)

2. Turn on the chiller bath at least an hour to prior to starting up the supercritical fluid extraction system. Set the chiller bath temperature to 5°C. (The chiller bath circulates a 50:50 mixture of ethylene glycol [antifreeze]:water.)

3. Once the chiller bath has stabilized at 5°C, open the regulator to the CO_2 cylinder. Make sure all valves between CO_2 pump and extraction chamber are open. Plug the extraction chamber into a wall receptacle. *[Note: The extraction chamber has a fan that runs continuously to prevent the circuitry from overheating. After extractions are completed and the extraction chamber has cooled to ambient temperature, the unit is unplugged from the wall outlet to minimize wear on the fan motor.]*

4. Turn on the extraction chamber, pump controllers, restrictor controller, and printer (fig. 2).

5. Set the extraction chamber temperature to the desired set point. *[Note: The extraction temperature must always be set manually. Although the SFE pump program includes an entry for temperature, this entry is merely for printer/display purposes.]* On the temperature control panel, press the "PV/SV" button to toggle between present value (actual temperature) and set value. To adjust the set value, select the "^" toggle under the desired decimal place. Press "^" once to highlight the place, then either press "^" or "ˇ" to adjust that decimal place to the desired value. To adjust another decimal place, select the "^" under that place to highlight it, then adjust as needed. When finished, press "ENT" to accept the new set value. Allow approximately 1 h for the temperature of the heating block to stabilize.

6. Calibrate the restrictor controller each time the unit is turned on. At room temperature (~21°C), the resistivity of the restrictor controllers should be calibrated to a value of 5.43. *[Note: In a typical capillary restrictor system, the controller is capable of independently regulating two restrictors, hence, controls for "restrictor 1" and "restrictor 2"are indicated. However, in this system, both controllers are needed to regulate the one manually variable restrictor. Both controllers must therefore be calibrated.]*

7. On the restrictor controller panel, press "calibrate" once to display the actual value. Press "+" or "-" until the value displayed reads 5.43. Press "calibrate" again to calibrate the resistor. Repeat this procedure for the other display.

8. After calibration, the restrictor controller displays will revert to displaying actual temperature values. Set the temperature on each controller to 100°C by depressing the "+" toggle.

Preparing the Extraction Vessel:

9. Once the extraction chamber temperature has stabilized at the desired setting, prepare the extraction vessel. If the vessel has not yet been cleaned, place all the vessel components (filters, vessel body, top and base) into a glass beaker. Under a fume hood, pour clean DCM into the beaker, covering all the components. Let the components soak for 1-2 min. Drain off the DCM into an appropriate hazardous waste container, and repeat the process again until the components have been rinsed three times with DCM. Cover the beaker loosely with aluminum foil, and allow the components to dry under the hood (~15-20 min).

10. Assemble a precleaned vessel base, fritted filter, and vessel body. Make sure that the "S" marked on the filter faces the sample. *[NOTE: The "S" is present merely to help the user keep track of which filter side faces the sample – the filter is equally effective in either orientation. However, if filters are used in multiple extractions, then it is important that the same side faces the sample during each extraction. If the alternate side is used, particles that were once entrained in the filter may be mobilized and become lodged downstream in the instrument plumbing, constricting flow.]*

11. Insert a small amount of glass wool (or quartz sand, if desired) into the base of the vessel. These materials reduce dead volume in the extraction vessel while also mitigating the potential for coal particles to clog the filter.

12. Weigh the prepared extraction vessel to a precision of 0.001 mg.

13. Carefully add the coal sample to the extraction vessel and reweigh the vessel assembly.

14. Add another layer of glass wool (or quartz sand, if desired) to the extraction vessel to minimize dead volume. Remove any particles from the threads at the top of the extraction vessel.

15. Add surrogate spikes, if desired, consisting of 10 µl each of the prepared aliphatic and aromatic surrogate solutions. To minimize the loss of semivolatile surrogate compounds, do not add the spikes until immediately prior to commencing an extraction.

16. Place a second fritted filter into the vessel cap. Make sure that the "S" stamped on the filter faces outward, so that the "S" will ultimately face the sample. Screw the vessel cap onto the assembly. Take the loaded, sealed extraction vessel (fig. 4) and proceed with sample extraction.

Conducting Extractions

Creating an Extraction Program

1. From the Extract Program menu (press the "extract pgrm" key if this menu is not currently displayed), press "C" to select the program option.

2. Press "1" to edit/view file.

3. Type the number of an existing file (to modify), or type a new number that will be used to refer to a newly created program.

4. Press "enter." At this point, several extraction program parameters will be displayed. (See instrument manual for further description.) To highlight a parameter, simply type the number corresponding to that parameter. If that parameter contains a setting with a numeric value, then type the desired numeric value and press "enter" to retain the desired value. If a highlighted parameter, such as refill mode, contains a text entry, then typing the number corresponding to that parameter will toggle through the possible options, such as manual refill versus auto refill.

5. When all desired changes to the new/modified program are completed, press "Store" to exit this menu and save the changes to the file number.

Running a Different Extraction Program

6. Press the "Recall" key.

7. Type "1" to select SFX1 File option. This option refers to extractions that will be conducted in chamber 1. *[NOTE: Although SFX2 is listed as an option, the system in its present configuration is unable to conduct extractions in chamber 2. The manually variable restrictor occupies both restrictor ports, and only extraction chamber 1 has access to the manually variable restrictor.]*

8. Use the keypad to type the number of the desired file (program). Press "enter" when finished.

9. Press "D" to revert to the previous screen. This action reverts back to the extraction program menu display.

10. Press "A" to start the extraction program. At this time, the pump will adjust to any changes, such as differences in pressure settings between the previous and newly selected extraction program. VERY IMPORTANT: This course of action will not change the temperature setting for the extraction. Any desired changes to the extraction temperature must be entered manually using the temperature controller on the extraction chamber.

Executing a Loaded Extraction Program

11. The conditions for the last extraction program used will be displayed on the system controller. The following extraction conditions are typically used in this study (program file #1):

Table 1. Typical SFE Conditions Used in This Study.

Sample mass	1.000 g (if using ground coal)
Extraction Solvent	100% CO_2 (no modifier)
Extraction Temperature	40°C
Extraction Pressure	100 bar
Step 1 – Static (no flow)	15 min
Step 2 – Dynamic (flow)	60 min (or until pump dispenses 110.4 ml of CO_2)

If extractions will be performed using the same program, then one generally needs only to press "A" under the "start F01" display to start the extraction. *[Note: The syringe pump was programmed to dispense a maximum of 110.4 ml of CO_2. This volume of CO_2, at 100 bars and 5°C in the pump head, was chosen so that the resulting solvent:sample ratio would be similar to the solvent:sample ratio obtained during parallel coal sample extractions using DCM in a Soxhlet extraction apparatus.]*

12. Set up the solvent trap. (This task can be performed while the system is executing a static extraction step.) Fill a small plastic bowl with a mixture of ice and water. Remove the 125-ml flask containing the hexane, that is, the solvent trap, from the refrigerator and set the flask into the ice-water bath.

13. Rinse the restrictor tip with DCM; collect the solvent runoff in a glass beaker, and transfer the solvent rinse to an appropriate hazardous waste container.

14. Place the solvent trap and bowl under the restrictor tip. Slowly raise the trap (bowl) and insert the restrictor tip into the flask. Make sure that the base of the restrictor tip is positioned below (~ 2-3 cm) the level of hexane in the solvent trap.

15. Place supports under the bowl to maintain an appropriate height with respect to the restrictor tip.

16. Unscrew the extraction chamber cap from chamber number 1. *[Note: The manually adjustable restrictor currently installed occupies both restrictor outlets, so extractions can only be performed using chamber 1.]*

17. Insert the nipple end (vessel top) of a fully loaded extraction vessel into the base of the extraction chamber cap. The vessel snaps into place, but continues to exhibit some range of motion.

18. Insert the cap-vessel assembly into the extraction chamber, and screw in the assembly until the cap is finger tight. DO NOT overtighten.

19. Press "A" under the "Start F01" display to begin the extraction.

20. At the beginning of the dynamic extraction step, the initial CO_2 flow rate will be very large, as the pump attempts to equilibrate with the changing flow conditions in the system. The flow rate will diminish

rapidly within the first 30 seconds. After this time, if the CO_2 is flowing too vigorously through the solvent trap –causing splashing– then turn the knob on the adjustable restrictor clockwise to limit flow and(or) achieve desired flow rate.

21. Periodically monitor the CO_2 flow rate through the restrictor. A flow rate in the range of 1.7-2.0 ml CO_2 min^{-1} through the restrictor is needed to dispense 110.4 ml of CO_2 by the end of the 60 min dynamic extraction step.

22. At the end of the dynamic extraction step (60 min or 110.4 ml of CO_2 dispensed), the extraction results will be sent to the printer. The supercritical fluid extraction unit will also vent the pressurizing fluid, and refill the syringe pump with CO_2 automatically (if the automatic refill option within the extraction method has been selected).

23. Slowly remove the solvent trap supports, and remove the flask containing the hexane and extracted hydrocarbons from the restrictor unit. Cap the flask and store at 4°C or proceed with "Preparing the Sample Extract for Subsequent Processing".

24. Once the supercritical fluid extraction unit has finished venting, the extraction chamber pressure will have decreased sufficiently such that the extraction vessel can be safely removed from the extraction chamber. Unscrew the extraction chamber cap-vessel assembly and remove from the unit. Set the assembly aside to cool, if necessary.

25. When cooled, detach the extraction vessel from the cap. [At this point another loaded extraction vessel can be attached to the cap and another extraction started immediately.]

26. Unscrew and remove the extraction vessel caps. Remove the glass wool, and transfer the extracted coal sample to a glass vial and archive, if desired.

27. Under a fume hood, use forceps to hold the individual vessel components and rinse each component three times with DCM to remove particulates and organic residues.

28. After rinsing, place the components in a beaker and soak in clean DCM for 5-10 min.

29. Drain off the DCM into an appropriate hazardous waste container, loosely cover the beaker with aluminum foil and let the vessel components dry under the fume hood. When dried, the vessel is ready for another extraction.

Preparing the Sample Extract for Subsequent Processing

Reagents Needed:

nitrogen (gas): grade 99.998% or better [use in-line filter to increase purity to > 99.999%]; used to evaporate solvent

30. Place the 125-ml flask containing the sample extract (in hexane) into an NEVAP turret and evaporate the sample extract under a gentle stream of nitrogen.

31. Evaporate the sample extract to a volume < 5 ml, and transfer via pipette to a 5-ml, graduated, glass centrifuge tube. Rinse the walls of the extraction flask three times with clean hexane, each time transferring the hexane rinse to the same centrifuge tube containing the sample extract. Bring the final solvent volume (sample extract plus three rinses) to 5 ml. (Place the centrifuge tube in the NEVAP turret and evaporate the extract to accommodate the hexane rinses, if necessary.)

32. Cap the centrifuge tube and store at 4°C. Commence the procedure for EOM determination, or the procedure for separating the extracted hydrocarbons into compound classes, as appropriate.

Extractable Organic Matter (EOM) Determinations

Preparing Aluminum Foil Sample 'Boats'

1. Wearing appropriate gloves to minimize contamination, cut aluminum foil into squares measuring roughly 2cm x 2cm in size.

2. Holding an aluminum foil square in your gloved hand, place the end of a 14/20 ground glass stopper in the center of the foil square and mold the foil around the end of the stopper. (These aluminum "boats" can then be ashed in a muffle furnace for 6 h at 450°C to remove remnant organics.)

Preparing the Sample

3. Remove sample extracts from the refrigerator ~ 1 h before analysis and allow the extracts to warm to room temperature.

4. Check the extract volume after samples have warmed to room temperature. If necessary, bring sample extracts up to full volume with the appropriate solvent:

- If determining EOM on a <u>total</u> (unfractionated) sample extract, then dilute the total extract to 5.00 ml with hexane.
- If determining EOM on the <u>aliphatic</u> fraction of a sample extract, then dilute the fraction to 1.00 ml with hexane.
- If determining EOM on the <u>aromatic</u> fraction of a sample extract, then dilute the fraction to 2.00 ml with 30D (30% v/v DCM in hexane).

5. If working with total (unfractionated) extracts, proceed directly to "EOM Determination". If using fractionated extracts, then first spike the fractionated extract with the appropriate internal standards and allow extracts to sit for ~ 1 h to homogenize. Proceed to "EOM Determination".

EOM Determination

6. Use a microbalance to measure changes in mass. Record values to a precision of 0.001 mg.

7. Tare the microbalance, then place an empty aluminum boat on the balance and record the mass. Do NOT tare! Transfer the weighed aluminum boat to a clean glass surface, such as a Pyrex baking dish, using forceps. Repeat this step for each sample.

8. In the first boat, pipette 50 µl of clean solvent. This boat will be used as a control.

9. Rinse the pipette thoroughly with solvent, and then proceed to pipette 50 µl of the first sample into the next boat. Repeat this process until all samples have been pipetted.

10. Wait 15 min, and then reweigh each sample (boat). Calculate EOM from the difference between this mass (at t=15 min) and the mass of the empty boat. Subsequent weights are used to monitor further changes in EOM and also to provide an indication as to the volatility of the EOM, such as the abundance of low-molecular weight hydrocarbons.

11. Wait another 15 min, and then reweigh all boats.

12. Wait another 30 min, and record a final weight for all boats. Depending on the amount and volatility of the EOM, some sample boats may still be losing mass. Dispose of used boats as hazardous waste using appropriate protocols.

13. Proceed to 'Separating the Extracted Hydrocarbons into Class Fractions,' as appropriate.

Separating the Extracted Hydrocarbons into Class Fractions

Reagents Needed (per sample extract)

All samples:

2.5 g neutral alumina, 5% deactivated with H_2O (Aldrich #199974, ~ 150 mesh)
2.5 g silica grade 62, 100% activated (Aldrich #243981, 200 mesh)
5.0 g silica grade 923, 100% activated (Aldrich #214477, 100-200 mesh)
~2.0 g activated copper powder (Fisher C434-500, ~300 mesh)

If collecting aliphatics and aromatics only:

25 ml [5+5+15] pesticide-grade (or better) hexane (plus additional hexane to prepare column)
60 ml [5+5+50] pesticide-grade (or better) mixture of 30% (v/v) DCM in hexane

If collecting aliphatics, aromatics, and polars:

25 ml [5+5+15] pesticide-grade (or better) hexane (plus additional hexane to prepare column)
50 ml [5+5+40] pesticide-grade (or better) mixture of 30% (v/v) DCM in hexane
35 ml [5+5+25] pesticide-grade (or better) mixture of 30% (v/v) methanol in DCM, if collecting polar fraction

Activation Procedure

1. Spread a thin layer (<0.5 cm) of substrate (alumina or either silica grade) on a clean, ashed Pyrex drying tray.

2. Set the loaded tray in an oven and bake out overnight. Bake the alumina at 200°C; both grades of silica are baked out at 180°C.

3. After the substrate has been baked (activated), transfer the substrate immediately to foil-covered clean, ashed glass jars and store in a drying oven at 110°C until needed. The substrates can be stored for several months in this condition.

4. At this point, all three substrates are 100% activated. Both silica grades are ready for use.

5. Immediately prior to use, deactivate the neutral alumina 5% with water. Into a wide-mouth flask, weigh out as much alumina as needed for that day's chromatographic separations. To the same flask, add enough organic-free (Milli-Q) water so that the mass of water added is 5% of the mass of the neutral alumina.

6. Cap the flask, and then tumble until the neutral alumina appears homogeneous and does not contain any lumps. At this point, the alumina has been deactivated 5% and is ready for use.

Copper Activation

8. To a large flask, add only enough copper powder for the day's chromatographic separations.

9. Under a fume hood, add enough trace-metal grade hydrochloric acid (HCl; full strength, ~38%) to cover the copper powder. Swirl the flask and let stand ~ 1min.

10. Pour off the HCl into an appropriate hazardous waste container. Add fresh HCl to the flask and repeat the process until the copper has been activated with three doses of HCl. Pour off the remaining HCl.

11. Add 'organic-free' water to the flask. Swirl the flask and let stand 1 min.

12. Pour off the water into an appropriate hazardous waste container, and add another dose of water to the flask.

13. Repeat the process (~ three times) until the HCl has been thoroughly rinsed from the copper powder. The pH of the water rinses can be checked to confirm that the copper powder has been thoroughly rinsed.

14. Add methanol to the flask, covering the copper powder. Swirl the flask and let stand 1 min.

15. Pour off the methanol into an appropriate hazardous waste container, and add another dose of methanol.

16. Repeat this process until a total of six doses of methanol have been added to the flask. Pour off the remaining methanol.

17. Add DCM to the flask, covering the copper powder. Swirl the flask and let stand 1min.

18. Pour off the DCM into an appropriate hazardous waster container. Add another dose of DCM to the flask. Repeat the procedure until the copper powder has been rinsed with three doses of DCM.

19. Fill the flask with DCM. Stopper the flask and set it aside until needed.

Preparation of Alumina/Silica Gel Columns for Chromatographic Separations

20. Assemble a 200-ml reservoir glass column on a stand. Rinse the PTFE stopcock with DCM and assemble. Place a beaker under the column.

21. Using a long glass rod, lightly pack a glass wool plug at the base of the column. Rinse down the reservoir and column walls with DCM to flush all the glass wool fragments to the column base. Open the stopcock to drain some of the DCM into the beaker. Leave a 1-cm high column of DCM atop the glass wool.

22. Add activated copper to the column using a pipette. Rinse down the reservoir and column walls with DCM.

NOTE: *Once the column packing procedure has begun, maintain a layer of solvent above the top of the column packing material.* ***Do NOT*** *let the top of the packed material go dry!*

23. Open the stopcock and allow some of the DCM to drain into the beaker. Check that no copper is washed into the beaker. (If copper accumulates in the beaker, the glass wool plug is packed too loosely. Use a glass rod to tamp down the glass wool plug, and continue.)

24. Continue to add copper until a band measuring ~ 1.0 cm thick has accumulated at the base of the column. Rinse down reservoir and column walls with DCM.

25. Add 2.5 g of 5%-deactivated neutral alumina to a clean beaker and make a slurry with DCM. Pour all of the slurry into the reservoir. (Continue to hold the beaker over the reservoir, and rinse the beaker thoroughly with DCM, if necessary.)

26. Open the stopcock to drain some of the DCM to help pack the alumina. Rinse down the reservoir and column walls with DCM.

27. Repeat steps 25 and 26 with 2.5 g of 100% activated silica grade 62, and then with 5.0 g of 100% activated silica grade 923.

28. Leave the stopcock open and allow the DCM to drain out of the column. When the solvent level drops below the top of the packed column, immediately add ~ 1 ml of hexane.

29. Allow the hexane to drain through the column. When the solvent level drops below the top of the packed column, immediately add another ~ 1 ml of hexane.

30. Allow the hexane to drain through the column. When the solvent level drops below the top of the packed column, immediately add 25 ml of hexane, and allow the hexane to drain through the column. Close the stopcock just before the solvent level reaches the top of the packed column.

Chromatographic Separations of Total Sample Extracts into Compound Class Fractions

31. Open the stopcock to drain out the last of the hexane rinse. As the solvent level reaches the top of the packed column, keep the stopcock open and transfer via pipette the entire sample extract (5.0 ml) from the centrifuge tube to the top of the column.

32. Fill the centrifuge tube with 5.0 ml of hexane while the sample extract is loading onto the packed column. As the solvent level reaches the top of the packed column, add 1-2 ml of this hexane rinse. Wait for the solvent level to drop to the top of the packed column, and then add another 1-2 ml of the hexane rinse. Repeat until the entire 5-ml hexane rinse has been added to the column.

33. As the solvent level reaches the top of the column, turn off the stopcock. Rinse off the base tip of the column with DCM and carefully remove the beaker from under the column. Replace the beaker with an appropriate receptacle, such as a 25-ml pear flask, to collect the aliphatic hydrocarbon fraction.

34. Fill a glass graduated cylinder with 15 ml of hexane. Slowly pour all the hexane into the reservoir.

35. Immediately open the stopcock to begin eluting the aliphatic fraction into the receptacle.

36. While the aliphatic fraction is eluting, fill the glass centrifuge tube with 5.0 ml of 30D. As the hexane level reaches the top of the packed column, pipette 1-2 ml of the 30D rinse onto the top of the column.

37. As the 30D level reaches the top of the packed column, add another 1-2 ml of the 30D rinse. Repeat this process until the entire 30D rinse has been added to the column.

38. Fill the glass centrifuge tube with ***another*** 5 ml of 30D. Add this second rinse in the same manner as described in steps 36 and 37.

39. After the entire second 30D rinse has been added, wait until the 30D level reaches the top of the packed column and then close the stopcock.

40. Remove the receptacle containing the aliphatic fraction and rinse down the base of the column with 30D. (Collect this rinse in a waste container.) Stopper the receptacle and store at 4°C or proceed to evaporate the sample fraction under a gentle stream of nitrogen.

41. Place an appropriate receptacle, such as a 50-ml pear flask, under the column to collect the aromatic fraction.

*If aromatics are the **only** remaining fraction to be collected (no polars), proceed to step **42**.*

*If **both** aromatics and polars are going to be collected, proceed to step **45**.*

42. (*aromatics only*) Fill a graduated cylinder with 50 ml of 30D. Slowly pour all the solvent onto the top of the column.

43. Open the stopcock and elute the aromatic fraction into the receptacle.

44. As the solvent level reaches the top of the packed column, close the stopcock and remove the receptacle containing the aromatic fraction. Stopper the receptacle and store at 4°C or proceed to evaporate the aromatic fraction under a gentle stream of nitrogen. **Proceed to step 55.**

45. (*aromatics and polars*) Fill a graduated cylinder with 40 ml of 30D. Slowly pour all the solvent onto the top of the column.

46. Open the stopcock and elute the aromatic fraction into the receptacle.

48. Fill the glass centrifuge tube with 5.0 ml of 30M. As the 30D level reaches the top of the packed column, pipette 1-2 ml of the 30M rinse onto the top of the column.

49. As the 30M level reaches the top of the packed column, add another 1-2 ml of the 30M rinse. Repeat this process until the entire 30M rinse has been added to the column.

50. Fill the glass centrifuge tube with ***another*** 5 ml 30M rinse. Add this second rinse in the same manner as described in steps 48 and 49.

51. As the solvent level reaches the top of the packed column, close the stopcock and remove the receptacle containing the aromatic fraction. Stopper the receptacle and store at 4°C or proceed to evaporate the aromatic fraction under a gentle stream of nitrogen. Place an appropriate receptacle, such as a 25-ml pear flask, under the column to collect the polar hydrocarbons fraction.

52. Fill a graduated cylinder with 25 ml of 30M. Slowly pour all the solvent onto the top of the column.

53. Open the stopcock and elute the polar fraction into the receptacle.

54. As the solvent level reaches the top of the packed column, close the stopcock and remove the receptacle. Stopper the receptacle and store at 4°C or proceed to evaporate the aromatic fraction under a gentle stream of nitrogen. **Proceed to step 55.**

55. In preparation for GC-MS analysis, evaporate the fractions under a gentle stream of nitrogen.

Evaporate the aliphatic fraction to a volume <1 ml, then transfer to a 2-ml glass centrifuge tube. Rinse the aliphatic fraction flask three times with hexane, each time transferring the hexane rinse to the 2-ml centrifuge tube. Dilute (or evaporate) the solvent (extract+three rinses) as needed to bring the solvent level in the centrifuge tube to a final volume of 1 ml.

Evaporate the aromatic fraction to a volume <2 ml, then transfer to a 2-ml glass centrifuge tube. Rinse the aromatic fraction flask three times with 30D, each time transferring the 30D rinse to the 2-ml centrifuge tube. Dilute (or evaporate) the solvent (extract+three rinses) as needed to bring the solvent level in the centrifuge tube to a final volume of 2 ml.

Evaporate the polar fraction to a volume <2 ml, then transfer to a 2-ml glass centrifuge tube. Rinse the polar fraction flask three times with 30M, each time transferring the 30M rinse to the 2-ml centrifuge tube. Dilute (or evaporate) the solvent (extract+three rinses) as needed to bring the solvent level in the centrifuge tube to a final volume of 2 ml.

56. Spike the aliphatic and aromatic fractions with 10 µl each of their respective internal standards. The spiked fractions are now ready to be analyzed for EOM content, if desired, and are ready for GC-MS analysis.

GC-MS Analysis of Extracted, Separated Hydrocarbon Classes

All analyses are conducted using an Agilent 6890 Gas Chromatograph (GC) interfaced with an Agilent 5973 Mass Selective Detector (MSD). The MSD is operated in the selective ion monitoring (SIM) mode during the PAH analyses; the MSD is operated in the full scan mode for aliphatic hydrocarbon analyses (Table 2). Samples are automatically injected, using an HP 7683 Injector, in the splitless mode, and the temperature program has been optimized to maximize resolution of the compounds of interest.

The aliphatic and aromatic hydrocarbon sample fractions are quantitatively analyzed. The polar sample fractions currently are only qualitatively analyzed (using the same program as that developed for the aliphatic fraction). The target aliphatic hydrocarbon compounds include *n*-alkanes ranging from nC_9 through nC_{34}, and the regular isoprenoids pristane and phytane. The target aromatic hydrocarbons include two-ring through five-ring PAHs and several alkylated homologues (Table 3). Approximate retention times for these compounds are also given in Table 3.

Aliphatic compounds are quantitated using the response of the m/z 57 ion fragment. Polycyclic aromatic hydrocarbon compounds are quantitated using the response of the major (primary) ion fragment (Table 3). The secondary ion fragment is used to confirm peak identity. In both sets of hydrocarbon analyses, external standards are run with each batch of samples to generate five-point concentration-response curves. Compounds used in the internal and external standards were obtained from several sources, including: Cambridge Isotope Laboratories, Inc. (Andover, MA, USA), Chiron AS (Trondheim, Norway), NIST (Gaithersburg, MD, USA) and Ultra Scientific (North Kingston, RI, USA). For example, all PAH samples and external PAH standards are spiked with 10 µl of an internal standard solution containing 4,000 ng μl^{-1} of each of six perdeuterated PAHs (Ultra Scientific; US-108N). The accuracy of the PAH concentration-response calibration curve is checked through analysis of NIST SRM 1491, which contains several of the target PAH compounds of interest.

Table 2. Gas Chromatograph-Mass Spectrometer Conditions for Hydrocarbon Analyses.

Analytes (Mode)	Aliphatics & Polars (GC-MS)	Aromatics (GC-MS)
Injector Temp	280°C; splitless	280°C; splitless
Injection Volume	1 µl	1 µl
Carrier Gas/ Flow Rate	He; 0.9 ml min^{-1}	He; 0.9 ml min^{-1}
Column	HP5-MS; 25m x 0.25mm x 0.25µm	HP5-MS; 25m x 0.25mm x 0.25µm
GC Oven Program	50°C, hold 1.50 min; ramp 10°C min^{-1} to 315°C, hold 15.00 min	50°C, hold 4.00 min; ramp 10°C min^{-1} to 150°C; ramp 6°C min^{-1} to 230°C; ramp 3°C min^{-1} to 300°C; ramp 10°C min^{-1} to 310°C, hold 15 min
MSD Conditions	Full scan, 50-550 amu	SIM mode (see Table 3 for time windows and list of target analytes)

Table 3. Time Windows for GC-MS Analysis of PAHs.

ANALYTE	SOURCE	PURPOSE	RT	Major Ion (m/z)	Secondary Ion (m/z)
Window I					
Naphthalene-d8	Ultra	ISTD	11.70	136	68
Naphthalene	NIST	target	11.74	128	127
1-Benzothiophene	other	target	11.88	134	89
1-Methylnaphthalene	NIST	target	13.42	142	141
2-Methylnapthalene	NIST	[uncertified]	13.66	142	141
Biphenyl-d10	Cambridge	SURR	14.55	164	162
Biphenyl	NIST	target	14.60	154	153
2-Ethylnaphthalene	other	target	14.81	141	156
1-Ethylnaphthalene	other	target	14.87	141	156
2,6-Dimethylnaphthalene	NIST	target	14.96	156	141
Acenaphthylene	NIST	target	15.64	152	151
1,2-Dimethylnaphthalene	other	target	15.71	141	156
Acenaphthene-d10	Ultra	ISTD	16.08	164	162
Acenaphthene	NIST	target	16.16	153	154
2,3,5-Trimethylnaphthalene	NIST	target	17.33	170	153
Fluorene	NIST	target	17.64	166	165
Window II					
1,2,5,6-Tetramethylnaphthalene	ChironAS	target	19.99	184	169
Dibenzothiophene	other	target	20.29	184	139
Phenanthrene-d10	Ultra	ISTD	20.65	188	94
Phenanthrene	NIST	target	20.71	178	176
Anthracene-d10	Cambridge	SURR	20.82	188	189
Anthracene	NIST	target	20.87	178	176
1-Methylphenanthrene	NIST	target	22.96	192	191
Window III					
3,6-Dimethylphenanthrene	other	target	24.19	206	205
Fluoranthene	NIST	target	25.00	202	101
Pyrene-d10	Cambridge	SURR	25.74	212	213
Pyrene	NIST	target	25.78	202	203
1,2,4-Trimethylphenanthrene	ChironAS	target	27.26	220	205
Benz[a]anthracene	NIST	target	30.79	228	229
Chrysene-d12	Ultra	ISTD	30.88	240	236
Chrysene	NIST	target	30.96	228	226
Window IV					
Benzo[b]fluoranthene	NIST	target	36.23	252	253
Benzo[k]fluoranthene	NIST	target	36.36	252	253
Benzo[e]pyrene	NIST	target	37.58	252	253
Benzo[a]pyrene-d12	Cambridge	SURR	37.76	264	265
Benzo[a]pyrene	NIST	target	37.83	252	253
Perylene-d12	Ultra	ISTD	38.20	264	265
Perylene	NIST	target	38.27	252	253
Indeno[1,2,3-cd]pyrene	NIST	target	43.61	276	138
Dibenz[a,h]anthracene	NIST	target	43.90	278	139
Benzo[ghi]perylene	NIST	target	44.78	276	138

Figures

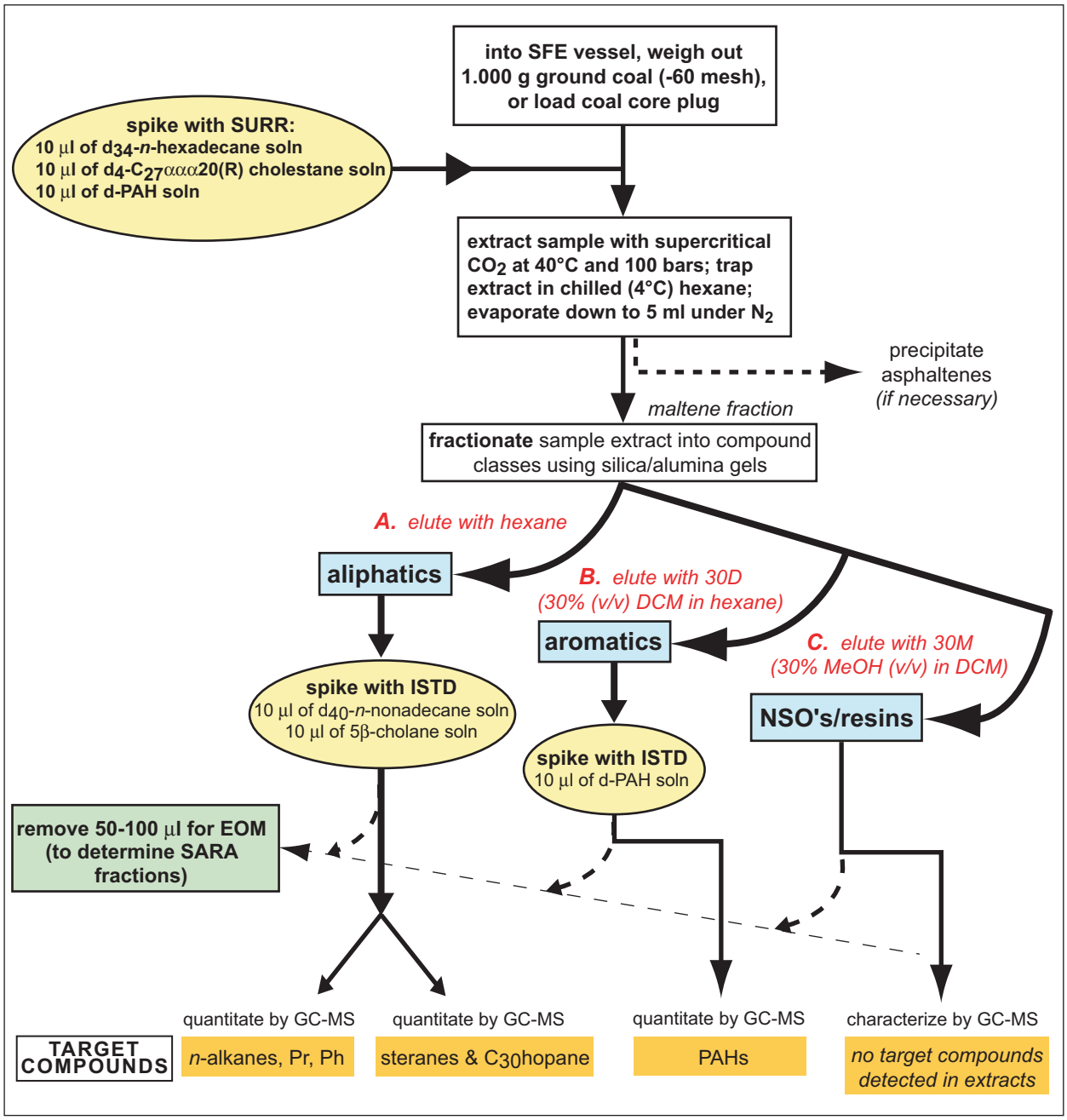
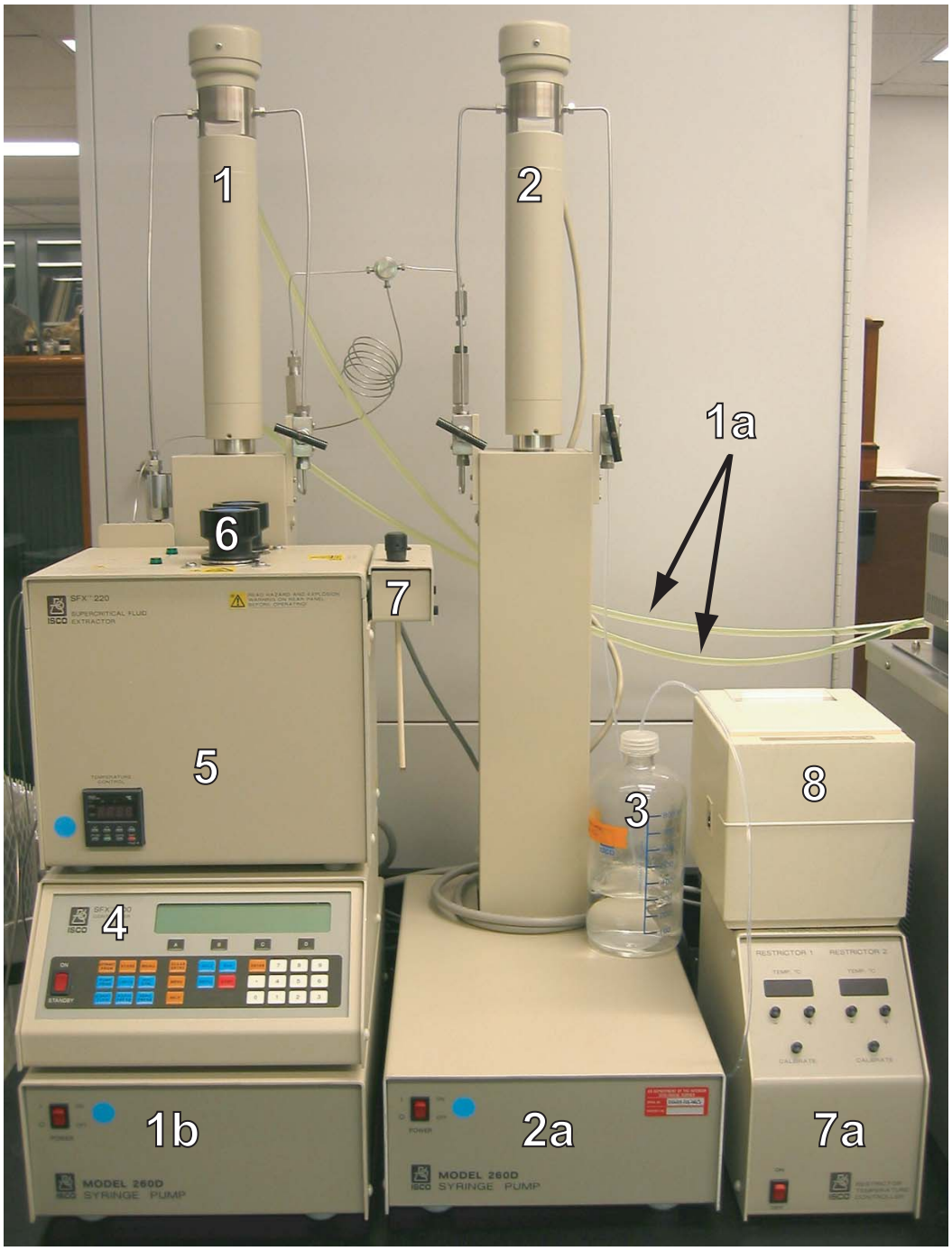


Figure 1. Flowchart depicting procedure for the supercritical CO_2 extraction of coal samples, with subsequent analyses of extracted hydrocarbons.



KEY TO NUMBERED ITEMS:

1:	CO_2 pump	5:	extraction chamber
1a:	coolant lines to CO_2 pump	6:	extraction chamber cap
1b:	CO_2 pump controller	7:	restrictor
2:	modifier pump	7a:	restrictor controller
2a:	modifier pump controller	8:	printer
3:	modifier reservoir		
4:	system controller		

Figure 2. Front view of the instrumentation (ISCO SFX220 Extractor and 260D Syringe Pumps) used to extract coal samples with supercritical CO_2.

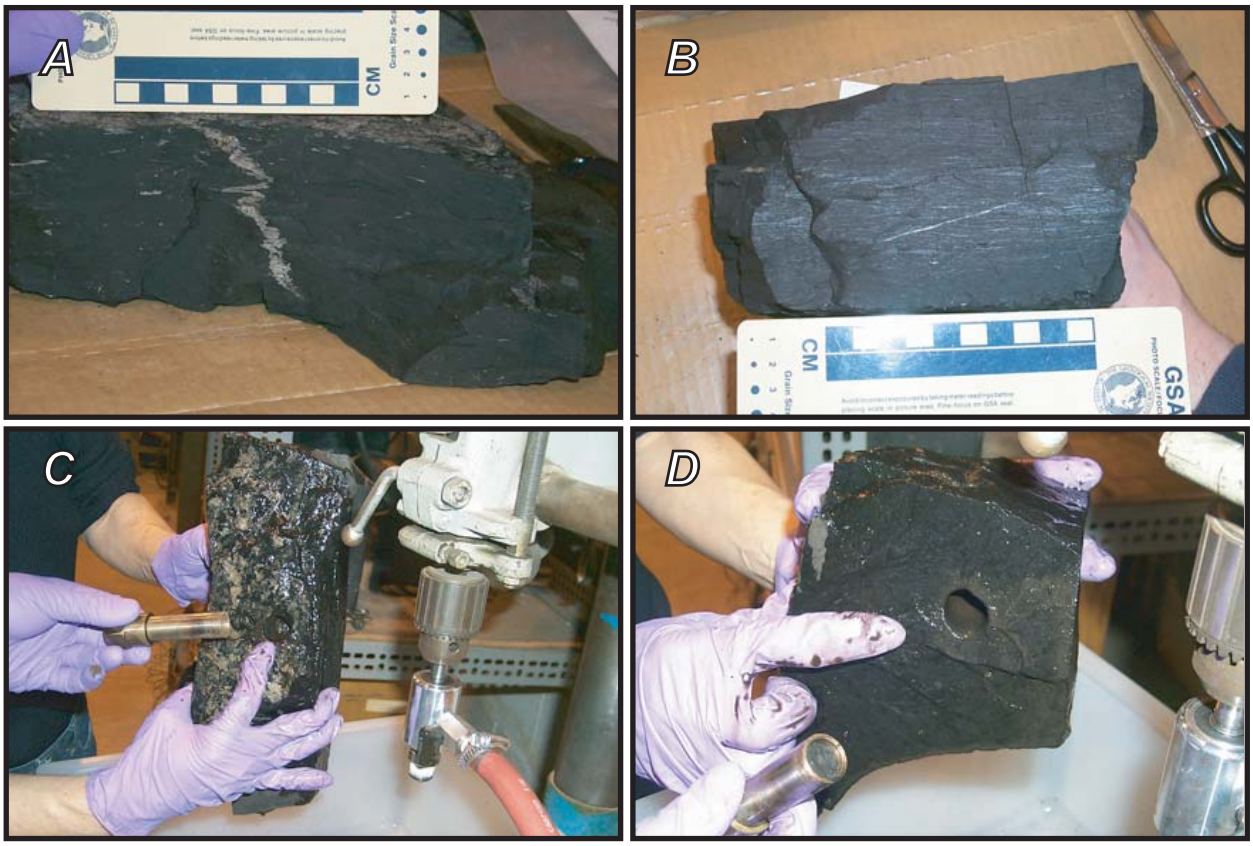


Figure 3. Collecting coal core plugs from channel bench samples: ***A*** View of sample OX-04-AB parallel to bedding; ***B*** View of sample OX-04-BB parallel to bedding; ***C*** View of core bit, sample OX-O4-AB, and drill press with water swivel attachment; and, ***D*** View of sample OX-O4-BB after coring [note the coal core plug present within the core bit]. Images courtesy of Peter Warwick (USGS).

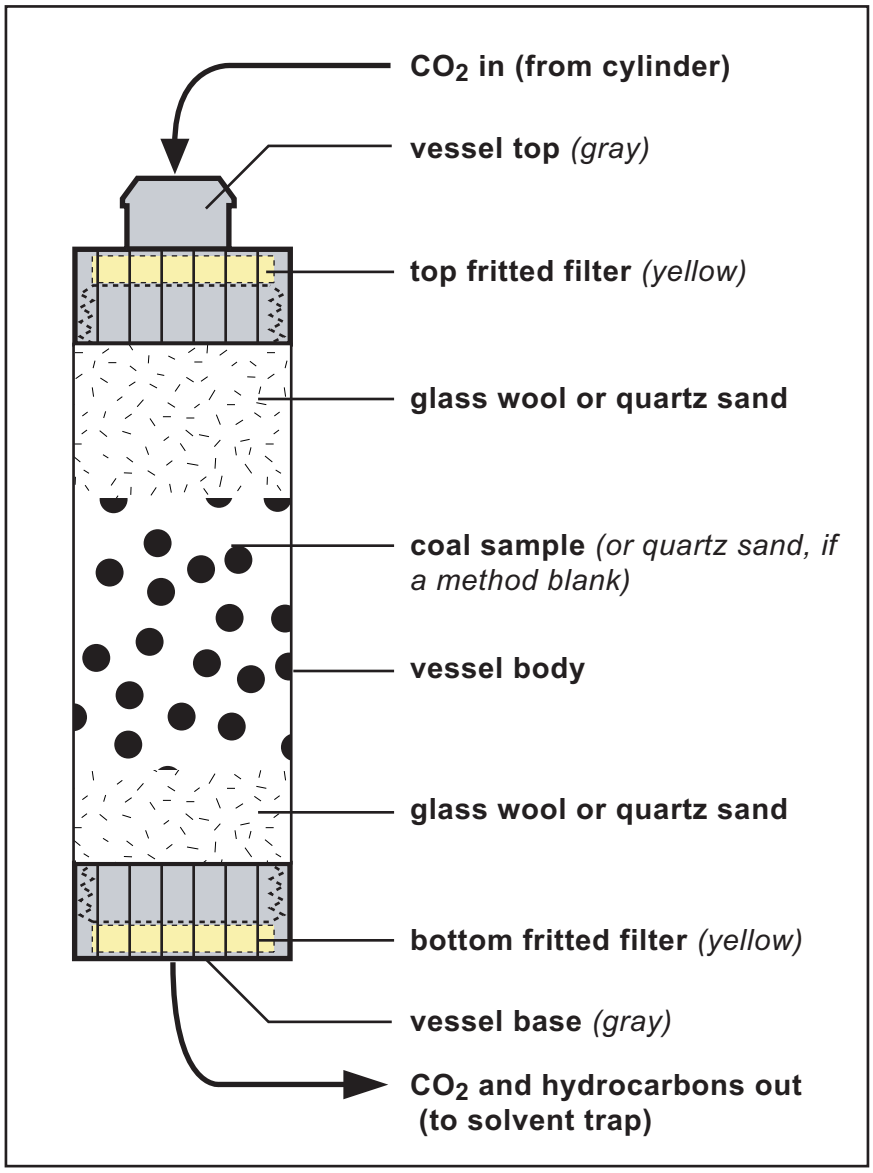


Figure 4. Schematic cross-section view of a loaded aluminum extraction vessel (10 ml capacity) used during the supercritical CO_2 extractions. Image not drawn to scale.

Appendix A. Job Hazard Analyses

The following Job Hazard Analyses (JHAs), excerpted from laboratory Chemical Hygiene Plans (CHPs), are provided below as indications of some of the hazards that may be present during these procedures, and as suggestions for the some of the precautions that could be taken to ensure the safe execution of the extraction and analysis procedures. These JHAs are by no means exhaustive, and any changes or modifications to the described extraction procedure may pose additional hazards not included below.

Job Activity: Supercritical Fluid Extraction

Description: Extract hydrocarbons from particulate coal/rock samples using supercritical fluids such as carbon dioxide with or without modifiers such as water, hexane, or brine.

Basic Job Steps	Hazards	Safe Job Procedure
1. Load supercritical fluid extraction instrument pumps with solvent of choice, and insert sample into extraction chamber.	**1. a)** Organic solvents are flammable and/or toxic.	**1. a)** Keep solvents away from open flames or other ignition sources. Collect sample extracts in a trapping device to minimize release of solvent vapors.
[Note: Extraction with carbon dioxide requires use of high-pressures (up to 7500 psi) and high temperatures (up to 150°C).]	**1. b)** Hot surfaces are present –may cause burns.	**1. b)** Wear insulated gloves when handling extraction apparatus.
	1. c) Risk of electric shock from water recirculator.	**1. c)** Check system for leaks before turning on electrical equipment.
	1. d) Liquid carbon dioxide may cause burns.	**1. d)** Wear insulated gloves when handling items.
	1. e) Extractions with carbon dioxide and other solvents with this SFE system require high pressures.	**1. e)** Wear goggles. Do not exceed recommended pressure for vessel.
	1. f) Compressed gas cylinders (carbon dioxide) are under high pressure.	**1. f)** Make sure gas cylinders are properly secured prior to use.
	1. g) Recirculating fluid from chiller contains ethylene glycol, which is toxic.	**1. g)** Avoid ingestion. Wear gloves and wash hands thoroughly following handling of the chiller and recirculating fluid.

Job Activity: Activate copper powder

Description: Activated copper powder is used in the preparative chromatography procedure to remove sulfide from sample extracts.

Basic Job Steps	Hazards	Safe Job Procedure
1. Load copper powder into glass filtration unit. Extract three times with each of the following (concentrated hydrochloric acid, organic organic-free water, methanol, and DCM). Store activated copper under DCM in centrifuge tube.	**1. a)** Copper powder and methanol are flammable.	**1. a)** Do not use around open flames or ignition sources.
	1. b) The organic solvents used are toxic.	**1. b)** Perform procedure under fume hood.
	1. c) Hydrochloric acid is corrosive.	**1. c)** Wear appropriate PPE, including gloves and goggles.

Job Activity: Preparative chromatography

Description: Separate extracted hydrocarbons into different compound classes using preparative chromatography.

Basic Job Steps	Hazards	Safe Job Procedure
1. Prepare glass column in DCM by loading with activated copper, alumina and silica gels. Switch solvent to hexane and load sample onto column. Elute different compound classes using different solvents, such as hexane (100%) and 30% DCM in hexane. Evaporate samples to ~ 1ml volume under a gentle stream of nitrogen.	**1. a)** Dust from silica and alumina gels is respiratory irritant.	**1. a)** Transfer gels carefully to minimize dust generation. Work under fume hood whenever possible.
	1. b) Solvents used are toxic and(or) flammable.	**1. b)** Do not use solvents around open flames or ignition sources. Perform fractionation under fume hood. Wear gloves and goggles.
	1. c) Cylinders of compressed gases (nitrogen) are under high pressure.	**1. c)** Ensure that gas cylinders are properly secured prior to use.

Job Activity: GC-MS Analyses

Description: Analyze extracted, separated hydrocarbon classes using GC-MS.

Basic Job Steps	Hazards	Safe Job Procedure
1. Load spiked sample extracts into GC-MS vials, and then into the autosampler. Conduct analyses.	**1. a)** High-temperature zones in GC-MS can cause burns.	**1. a)** Use caution, wear insulated gloves when necessary.
	1. b) The organic solvents used are toxic and(or) flammable.	**1. b)** Attach vent lines to convey exhaust gases to fume hood. Avoid sources of ignition.