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| **Brueckner Lab-Specific Standard Operating Procedure (LSOP):****CombiFlash®** |
| **Principal Investigator(PI):** Christian Brueckner |
| **Building:** Chemistry | **Lab(s) Covered by LSOP:** R413/R415 |
| **Department:** Chemistry | **Lab Phone Number(s):** 6-6596/6-6598 |
| **SECTION 1 – HAZARDOUS CHEMICAL(S) or PROCESS(ES) and HAZARDS INVOLVED** |
| The CombiFlash uses solvents under pressure to chromatographically separate compounds on pre-packed columns. The CombiFlash is an electrical machine also involving solvents – electrocution risk* The CombiFlash involves the use of volatile solvents that are flammable and pose fire/explosion risks.
* Other hazards associated with the solvents involved:
	+ Skin or eye irritation
	+ Skin defatting or dermatitis from prolonged or repeated skin exposure
	+ Central nervous system depression
	+ Reproductive and fetal effects
	+ Chronic toxic effects, such as liver or kidney effects, from inhalation of solvent vapors
* Hazards associated with the compounds to be separated
* Pressurized liquids can cause physical eye damage if hit by a high-pressure stream
* Silica gel – dust inhalation hazard
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| **SECTION 2 – ADMINISTRATIVE CONTROLS** |
| * Anyone using the chemicals and procedures described herein needs to have undergone the annual EH&S [Chemical Hygiene Training](http://www.ehs.uconn.edu/Chemical/?p=training)
* Be aware of the applicable safety data sheets (SDS): <http://www.msds.com>
* [Working Alone](http://policy.uconn.edu/2012/07/30/working-alone-policy/) is not permitted when using chemicals or processes described in this LSOP
* No one is allowed to use the equipment without going to a training session with the responsible person; documentation of training is required.
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| **SECTION 3- ENGINEERING CONTROLS** |
| * The CombiFlash should be located at a well-ventilated location
* The waste container should be placed within a secondary container
* Assure all electrical cords are in impeccable conditions
* Assure that all fittings are compatible with, and tightly to the inlets they are connected to
* Keep the lids of all reservoir solvents closed to prevent solvent evaporation
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| **SECTION 4 – WORK PRACTICES** |
| Researchers must make sure the amount of solvent is sufficient enough for completion of the experiment. Prior to each run, the CombiFlash system should be washed for a minimum of 10 min with the initial solvent composition of the upcoming run.All solvents stored on the solvent reservoir must be labeled regardless of whether the solvent is in use.* Avoid inhaling silica gel powder when preparing the cartridge by using a fume hood.
* Take special care to ensure the injection valve assembly is locked when starting a run. (If not, the machine will not automatically stop and solvent/product will overflow and spill.) In addition, if the solvent line is not screwed on properly to the injection valve assembly, there is a risk of flooding the machine.
* It is good precaution to remain close-by during a run, especially during the beginning to ensure the machine collects the fractions properly.
* Minimize the amount of solvents sitting outside the solvent cabinet
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| **SECTION 5 – PERSONAL PROTECTIVE EQUIPMENT (PPE)** |
| At a minimum, a lab coat, nitrile gloves, long pants, closed-toed footwear, and chemical safety glasses that meet ANSI standard Z-87.1 must be worn. |
| **SECTION 6 – STORAGE** |
| Make sure the instrument is fully switched off when not in use, and all solvent containers firmly closed.  |
| **SECTION 7 – SPILL AND ACCIDENT PROCEDURES** |
| * Any solvent spill should be handled in the manner outlined in that chemicals safety data sheet or chemical hygiene plan.
* In general, all affected areas should be rinsed under cold water for a minimum of 15 minutes
* Contaminated clothing should be immediately removed to prevent prolonged exposure to irritant.
* In case of spills of large quantities of solvent (>500 mL), evacuate the lab and consider your next move (911 or waiting until the solvent has evaporated, or a clean-up procedure, depending on the hazards the solvent poses
* Turn off all sources of ignition – push the red emergency shut-off button near the exit.

**Report any incident to the PI and fill out the** [**accident form**](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwiF3bPe1dPXAhVRRN8KHX4wDf4QFggmMAA&url=https%3A%2F%2Fchemistry.uconn.edu%2Fwp-content%2Fuploads%2Fsites%2F1259%2F2015%2F09%2FIncident-Report-Form.doc&usg=AOvVaw3Uov8IQ2Z-Kan) |
| **SECTION 8 – FIRST AID PROCEDURES** |
| * First Aid should be administered in a manner consistent with the spilled chemical’s safety data sheet.
* In general, all affected areas should be rinsed under cold water for a minimum of 15 minutes.

**Report any incident to the PI and fill out the** [**accident form**](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwiF3bPe1dPXAhVRRN8KHX4wDf4QFggmMAA&url=https%3A%2F%2Fchemistry.uconn.edu%2Fwp-content%2Fuploads%2Fsites%2F1259%2F2015%2F09%2FIncident-Report-Form.doc&usg=AOvVaw3Uov8IQ2Z-Kan) |
| **SECTION 9 – WASTE MANAGEMENT** |
| * A waste container should be situated next to the CombiFlash, inside of a secondary waste container.
* All waste must be labeled with “Hazardous Waste” stickers or tags, use full chemical names to describe the waste (i.e., no chemical abbreviations or symbols), be stored in sturdy containers with tight-fitting caps or lids, and be stored alone or with other compatible chemicals
* Hazardous wastes must be stored at or near a green “Satellite Accumulation Area” sign prior to disposal by EHS. Once the containers are 80% filled, fill our EH&S chemical [waste pickup form](http://ehs.uconn.edu/Regulated%20Waste%20Management/index.php)
* The [Chemical Waste Disposal Manual](http://ehs.uconn.edu/Chemical/ChemWasteDisp.pdf) must be used as a reference
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| **SECTION 10 – DECONTAMINATION PROCEDURES** |
| Water, soap to remove residues and dust – be careful not to use acetone etc. on plastic parts |

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| **SECTION 11 – SPECIFIC PROCEDURE** |
| *Cartridge Preparation* * Fill approximately half of the cartridge with silica gel powder in the hood, taking care to avoid inhaling it.
* Transfer your product using a glass bulb pipet to the cartridge by spreading the product evenly over the packed sponge filter. Rinse the round bottom flask that contained your product with DCM as much product as possible. Finally, rinse sides of the cartridge to ensure all of your product reaches the filter. Insert a white sponge filter to the top of the silica gel using the solid, block plunger, making sure that there are no air spaces in between the filter and the gel powder.
* Clean the CombiFlash injection valve assembly with kimwipe using acetone. Once the valve assembly dries, push it through the cartridge so that there is no space between the tip of the injector and the filter. Lock the injection valve assembly by turning the top clockwise until there is a click. (It is locked when a “cross” is made between the metal injection valve assembly and the plastic lips of the cartridge on the underside of the valve assembly.
* Place the locked cartridge-valve assembly upside-down by gently pushing the solvent line through the metal ring located on the adjustable flash column mount and balancing the cartridge against the metal ring.
* On the left-hand corner of the computer screen, either select File, “New” (if planning to construct your own solvent gradient) or select “Open” and “extract” (if planning to extract a previous gradient). Save the new file under desired name.
* Select Tools, Manual Control, and then “air purge cartridge” while the cartridge is still upside-down for 5-10 minutes. (The program will not time it.) In the meantime, prepare the fraction collection tubes by inserting new 8mL glass collection tubes into the rack.
* When purge is finished, click “stop” and turn the locked cartridge-valve assembly upright, and screw the tip of the cartridge into its appropriate metal fitting.

*Column Preparation** Selecting the appropriate size and type of column is critical.
* Remove the two red caps from the selected column and insert by raising the adjustable flash column mount followed by appropriate fitting of the column. Make sure both the cartridge and column are screwed in tightly.

*Computer Program Set-up** After exiting air purge, return to the main screen.
* On the main screen, click on “Tools”, then “manual control” to change the percentage of solvent B or to change solvents altogether.
* To create or edit the gradient method, click on “Method editor’’, then you may manually construct your own gradient by selecting the curve on the gradient graph and dragging the curve to generate the desired slope.
* Under “Peak Detection”, select “details” for each wavelength. In the same window, ensure “slope-based” and “threshold” are selected.
* Still under Method Editor, ensure that the flow rate, run length, solvents, and column size are correct.

*Initiating a Run* * Select the large “play” button.
* Enter the appropriate information for Loading Technique. (For our purposes, the loading technique is solid). Also, ensure that the machine has detected the correct collection tube rack.
* You can access your run live, or at any time by internet searching the IP address: <https://www.youtube.com/watch?v=Ym4DyDkAhyg>
* During a run, the gradient can be held constant by selecting “pause” button. The run can be completely stopped by selecting the “stop” button. If CombiFlash system does not begin to collect fractions when it needs to, click “collect all” so that it will immediately collect.

*Completing a Run** After the run is complete, (and before running a subsequent experiment), select “fast forward” to air purge the cartridge before unlocking and separating the injection valve assembly from the cartridge.
* Dispose wastes appropriately. Clean the plunger with a Kimwipe.
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| **SECTION 12A. APPROVAL** |
| I have reviewed, understand and agree to follow this lab-specific standard operating procedure (LSOP) for the use of the Teledyne CombiFlash system*.* Failure to follow this LSOP or lab-specific training guidelines is a violation of the [*University Health & Safety Policy*](http://policy.uconn.edu/2011/05/19/health-and-safety-policy/) and [*University Code of Conduct*](http://policy.uconn.edu/2011/05/17/employee-code-of-conduct/).Further approval and/or review of this LSOP by the PI/Supervisor is required if any of the following events occur:* A significant change in amount (i.e., doubling of the scale of reaction) or substitution of the chemicals in the procedure is planned
* A major change in the agreed-upon experimental set-up is planned (heating instead of room temp, etc.)
* Any signs of a failure in safety design or equipment are observed
* Any signs or symptoms of a chemical exposure to any personnel are observed
* Unexpected and/or potentially dangerous experimental results occur (e.g., fire, uncontrolled buildup of heat and/or pressure, etc.)
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| **Researcher Name/Signature** | **Trainer Name/Signature** | **Training Date** |
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| **SECTION 12B. PRINCIPAL INVESTIGATOR CERTIFICATION** |
| I approve the contents of the lab-specific standard operating procedure listed above. |
| **PI Signature:** | **Date:** |
| **A HARD OR ELECTRONIC COPY (https://bruckner.research.uconn.edu/safety-resources/) OF EACH LAB-SPECIFIC STANDARD OPERATING PROCEDURE MUST BE READILY AVAILBALE IN THE LAB.** |