# **Standard Operating Procedure (SOP) Title: Flash Column Chromatography**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Assessor: | Joshua Linfoot | | | Location of work: | MSRH 502 |
| Principal Investigator: | | | Prof Alan Spivey | | |
| Date of approval: | | 13/09/2021 | | Date for review: | 13/09/2022 |

## **Justifying the hazards:**

Column chromatography in Chemistry is a method used to purify individual chemical compounds from mixtures of compounds. The technique uses an organic solvent as a mobile phase.

|  |  |  |  |
| --- | --- | --- | --- |
| Identify hazards with specific risk assessments and a College or a departmental approval process | | | |
| [Ionising radiation sources](https://www.imperial.ac.uk/safety/safety-by-topic/laboratory-safety/) | ☐ | [Biological sources](https://www.imperial.ac.uk/safety/safety-by-topic/laboratory-safety/) (microorganisms, human/animal tissues, plants) | ☐ |
| [Class 3R, 3B or 4 Lasers](https://imperiallondon.sharepoint.com/sites/fons/faculty/safety/lasers/SitePages/laserhome.aspx) | ☐ | [Offsite work](http://www.imperial.ac.uk/safety/safety-by-topic/off-site-working/) | ☐ |
| Confirm if [Lone working](https://www.imperial.ac.uk/safety/safety-by-topic/lone-working/) is permitted with this SOP? ☐ If it is permitted, describe the control measures for lone workers: | | | |

## **Preparing for the SOP:**

* **DON’T** use silica gel outside the fume cupboard. **DON’T** breathe in silica dust.
* **DON’T** use cracked and chipped glassware. Refer to SOP for Use of Glassware.
* **DON’T** start the procedure before familiarision with SOP for Compressed gasses or SOP for Schlenk Line depending on the source of N2.
* **DO** use a designated waste route for TLC plates.

## **Procedure:**

# **Before the procedure:**

1. Choose eluent, columns size and volumes of fractions to be collected. Use a column with a sintered filter embedded at the bottom of it.
2. Close the tap at the bottom of the column and place the flash column in a fume cupboard where there is access to N2 gas.
3. Make a slurry of the silica and eluent in a large beaker, stirring well with a glass rod. Slowly pour the slurry into the column allowing it to run gently down the walls until a few cm below the lower end of the ground glass joint.
4. Apply air pressure using the fitted pump to fully compress the gel - you will notice when the upper edge of the dispersed silica stops moving downwards. It may be necessary to run more eluent through the column while applying a steady air pressure until all the gel is evenly wet with the eluent and no part of the column is hot. It is recommended to use a t-adaptor with a stopper on one end, which can be shot out to release excess gas if present, preventing the breakage of the column.
5. Excess eluent will continue to drip out of the column until the upper level of the silica gel is reached.

# **During the process:**

* Application of the sample absorbed to stationary phase:
  1. Dissolve the sample in a suitable solvent in a suitably sized round bottom flask
  2. Add a small amount of dry silica (usually around the same mass as the crude mixture being purified).
  3. Evaporate the solvent (see separate SOP for Rotavapor). This process requires special attention and should be supervised by an experienced user.
  4. Scrape the silica mixture away from the sides of the flask with a bent spatula and apply the dry residue on top of the packed column.
* Eluting the column:

1. Ensuring the tap is closed, fill the column with the eluent once more, allowing the solvent to run gently down the walls of the column so as not to disturb the top of the silica bed.
2. Put a suitably sized beaker under the column, open the tap and apply N2 pressure so that the liquid volume decreases with a rate of roughly 1 mm/s. It is recommended to use a t-adaptor with stopper on one end, which can be shot out to release excess gas if present, preventing the breakage of the column.
3. Collect fractions with the recommended volume.
4. Elute the column until the liquid is a few cm above the gel surface. Release the pressure and close the stopcock. Check the fractions. If all the wanted products have departed the column, the elution can be stopped. If not, the column can be refilled with more eluent, and the elution continued.

* Examining fractions and evaporation of pure fractions:

1. Check the collected fractions using TLC (See SOP for TLC).
2. Fractions pure on TLC with regard to the wanted product, are combined.
3. If necessary, filter the solution to remove silica particles etc.
4. Evaporate the solution in a weighed round bottom flask (see separate SOP for Rotavapor).

# **After the procedure:**

1. Disassemble the column and dispose silica and TLC as silica waste.
2. Make sure you **leave the fume cupboard and working area clean**.
3. Wash glassware following SOP for Glassware.

## **Disposal:**

If any chemical waste is produced, ensure it is disposed of via the appropriate chemical waste route. All glassware should be cleaned, or disposed of following SOP for Glassware.

## **Personal Protective Equipment (PPE):**

Lab coat, appropriate gloves, safety glasses

## **Risk Analysis of SOP and emergency procedures:**

(In addition to [Safe Lab Practice](https://imperiallondon.sharepoint.com/sites/fons/faculty/safety/SitePages/Basic%20Laboratory%20Rules%20for%20All%20Laboratories%20in%20FoNS.aspx))

### **Always remember to include fire associated risks and control measures where appropriate**

|  |  |  |  |
| --- | --- | --- | --- |
| Hazard | Raw risks | Current control measures | Residual risk  (Low/Med/High) |
| Glassware and glass parts | Cuts and splinters from broken glass | Visually inspect glassware for cracks and other defects before and after use. If glassware damaged arrange for repair or dispose of. | Low |
| Glassware under pressure | Explosion | Always work in a fume cupboard with a lowered sash.  Visually inspect associated equipment (e.g. glassware and Schlenk line) for cracks and other defects before and after use.  If a gas cylinder is used, open the regulator slowly and under control. | Low |
| Hazardous materials | Exposure to hazardous reagents via inhalation or skin contact | No working with hazardous reagents outside of the fume cupboard.  Always wear appropriate PPE.  Ensure the fume cupboard and sample preparation area is cleaned after each use.  (Include hazards and controls of associated reagents in this or separate risk assessment) | Low |

|  |  |
| --- | --- |
| **Additional control measures to minimise residual risks** | **Implementation date** |
| Use a t-adaptor with a stopper on one end, which can be shot out to release excess gas if present, preventing the breakage of the column. |  |

|  |  |
| --- | --- |
| **Who may be harmed** | |
| Staff / students ☒ | Cleaners / Engineers ☒ |
| Supporting staff ☒ | Others (specify): |

|  |
| --- |
| **Emergency procedures** – describe the response(s) required by the user and lab members |
| Clear up **broken glass** using dustpan and brush, tweezers or other suitable equipment to prevent exposure to the glass then place into the appropriate waste bin (clean or contaminated glassware).  If anyone is injured while using the equipment contact a first aider.  If any **cuts or exposures** to hazardous substances, ensure affected area is held under running water for at least 15 mins and the wound is encouraged to bleed, ask for first aid assistance. If water is not available use alcohol free wipe from the First Aid Kit and dress the wound. Seek further medical attention if required.  **Chemical spills, risks specific to hazardous substances** - dependant on the nature of the chemical. Specific procedures will be outlined on an individual basis on the relevant COSHH form.  (Include emergency procedures associated with the use of hazardous substances if relevant) |

|  |
| --- |
| Recommended trainings and records: |
|  |

|  |  |
| --- | --- |
| List of individuals competent to demonstrate safe work practice and train others (level 1 trainers): | Names of those that have been trained and can work unsupervised (level 2) and date training completed: |
|  |  |
|  |  |