C18 Reversed-Phase Silica Gel Flash Chromatography – A Visual Walkthrough

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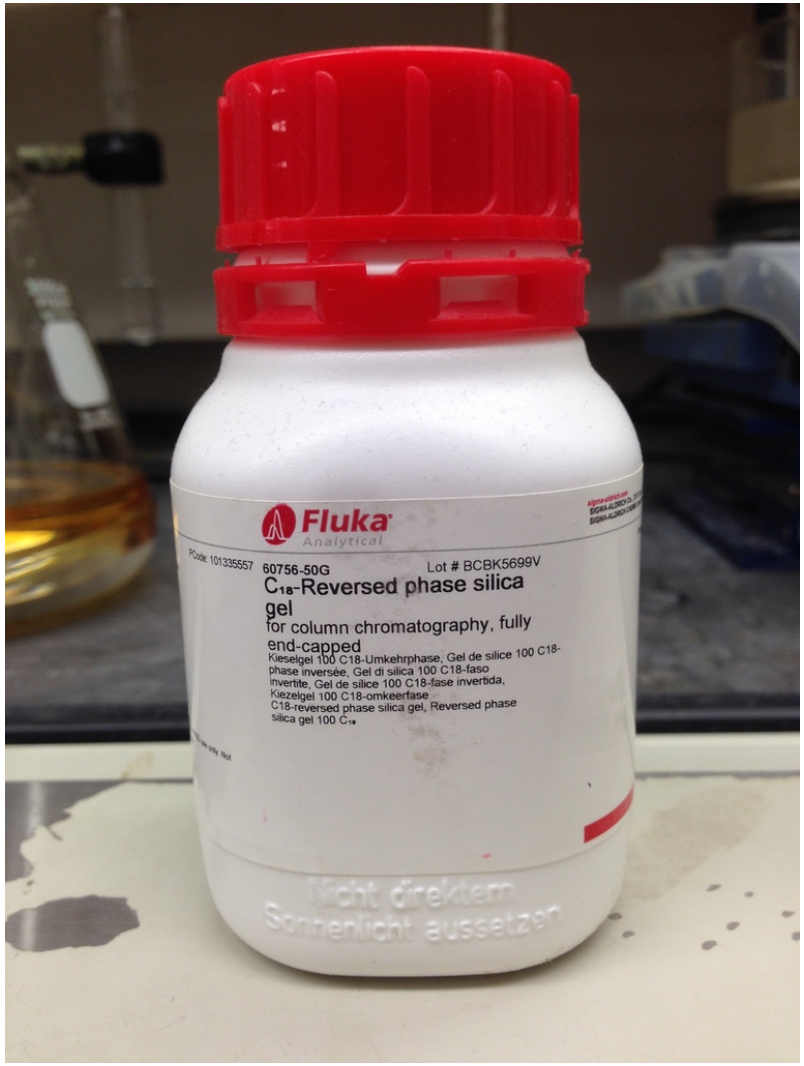
June 11th, 2015

The Stationary Phase

- Fluka 60756-50G
- C_{18}-Reversed phase silica gel
  - For column chromatography, fully end-capped
  - 15-25 um particle size
  - 100 Å pore size

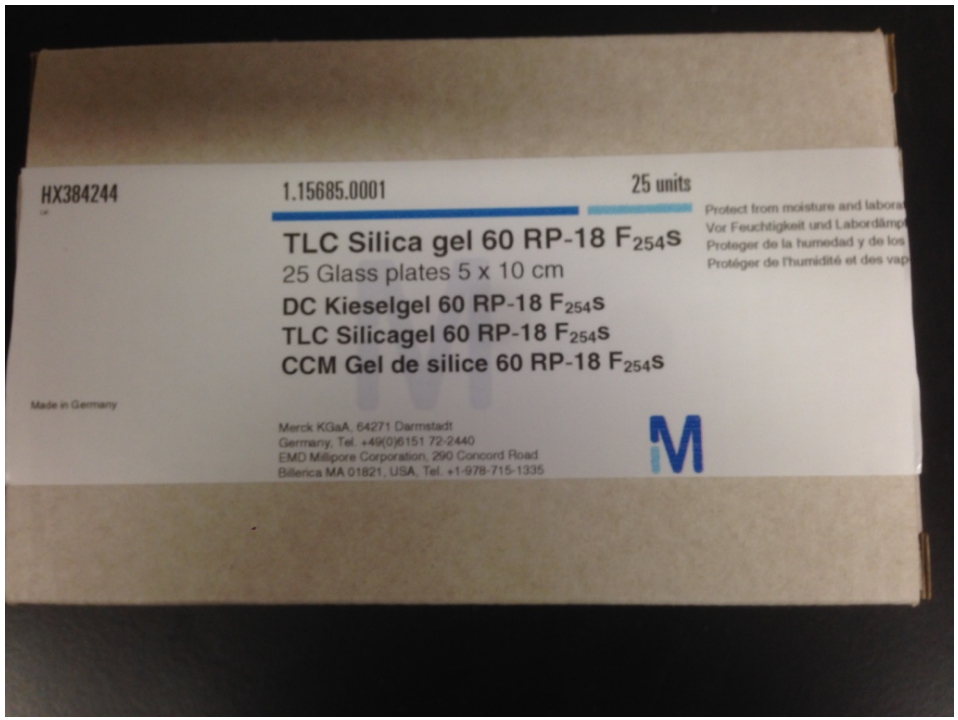


Price Comparison

- C18 Reversed-Phase SiO_2 – Fluka (Sigma-Aldrich)
  - $250 / 50 g
- Normal-Phase SiO_2 – Grace DAVISIL (Fisher)
  - $393 / 25 kg
    - $0.79 / 50 g

C18 RP TLC Plates

- EMD Millipore 15685
- TLC Silica gel 60 RP-18 $F_{254}s$
- 25 count
  - 5 x 10 cm



Comparing Price

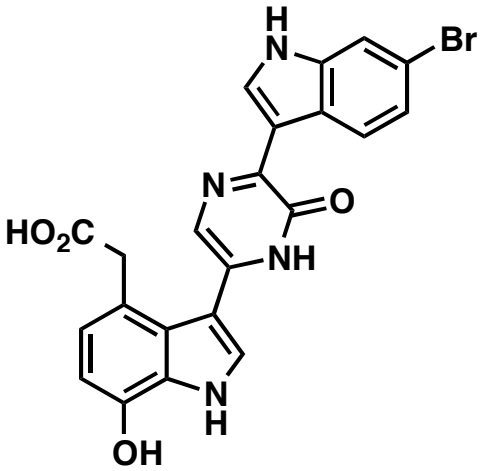
- C18 Reversed-Phase TLC – EMD Millipore (EMD)
  - $248 / 25 (5 x 10 cm) plates
  - $9.90 / 1 (5 x 10 cm) plate
- Normal-Phase TLC – EMD Millipore (EMD)
  - $150 / 25 (20 x 20 cm) plates
  - $0.75 / 1 (5 x 10 cm) plate

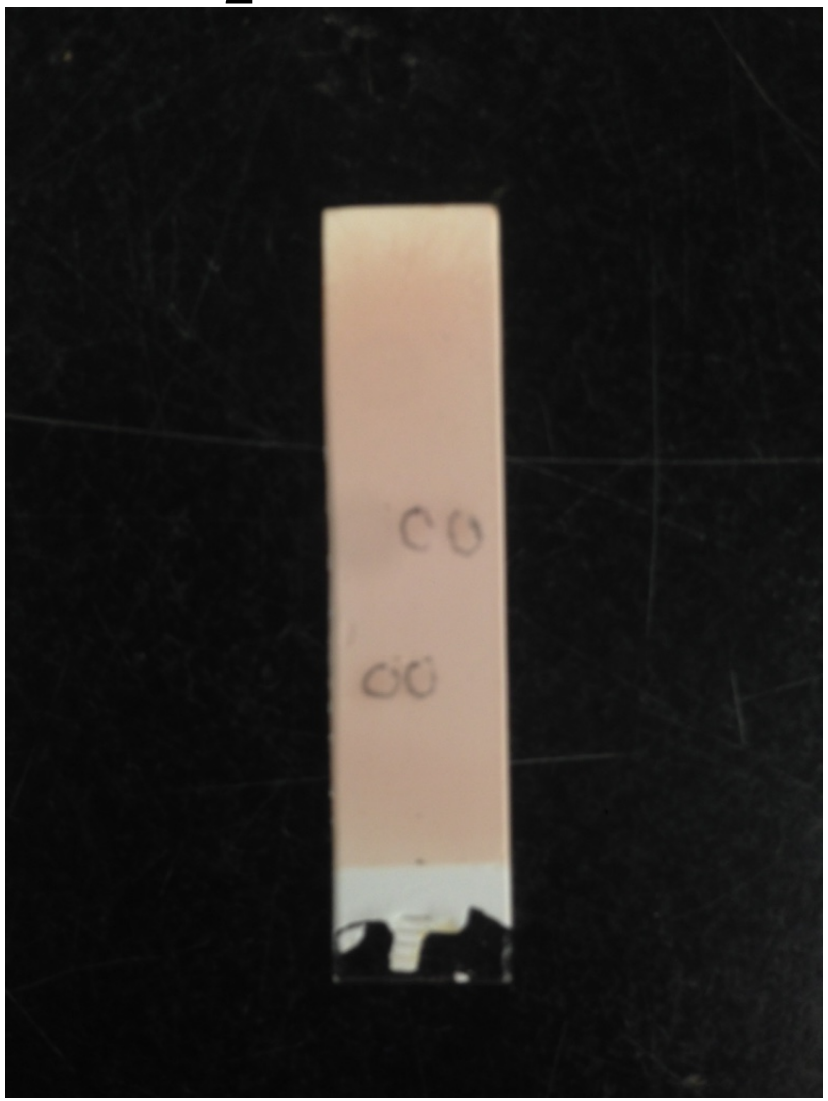
TLC Plates

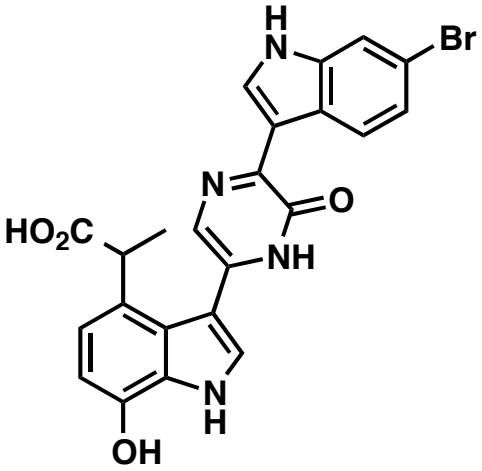
- Cut into 1 cm x 5 cm plates
- Will take longer to run that normal-phase TLC plates
- Stain just like NP TLC
  - Dry mobile phase from plate very well before developing

Example of TLC

- 50:50 MeCN:H_2O w/ 0.1% TFA

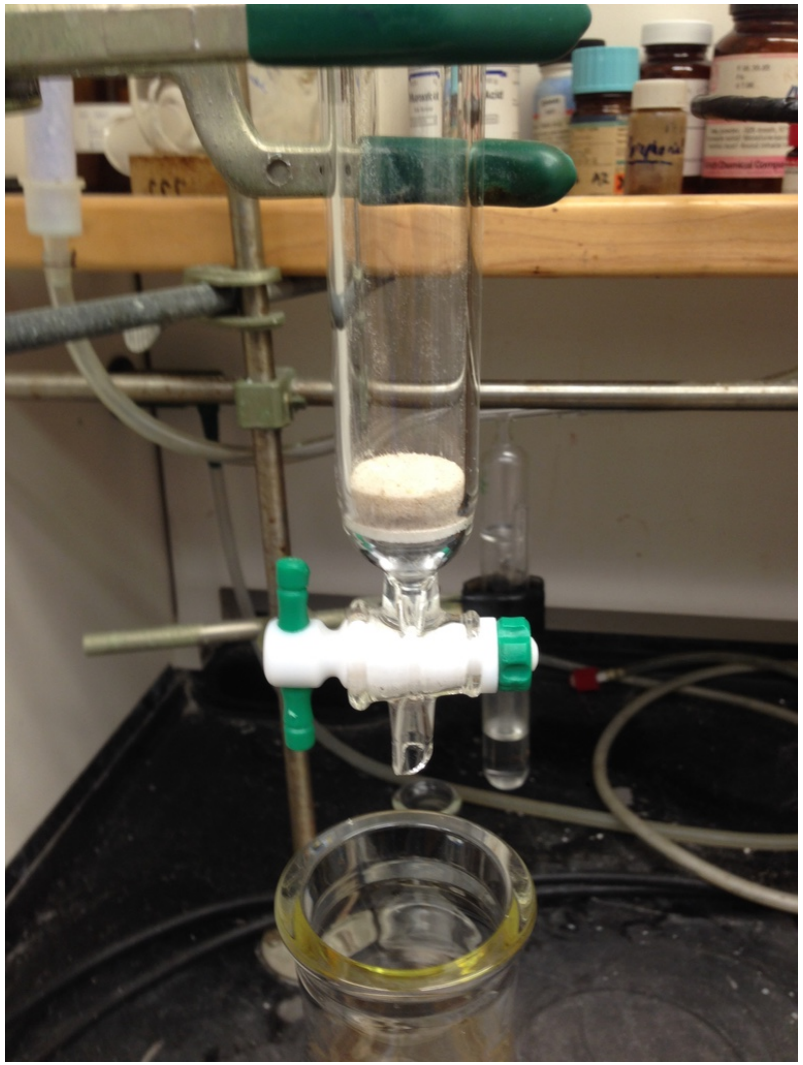






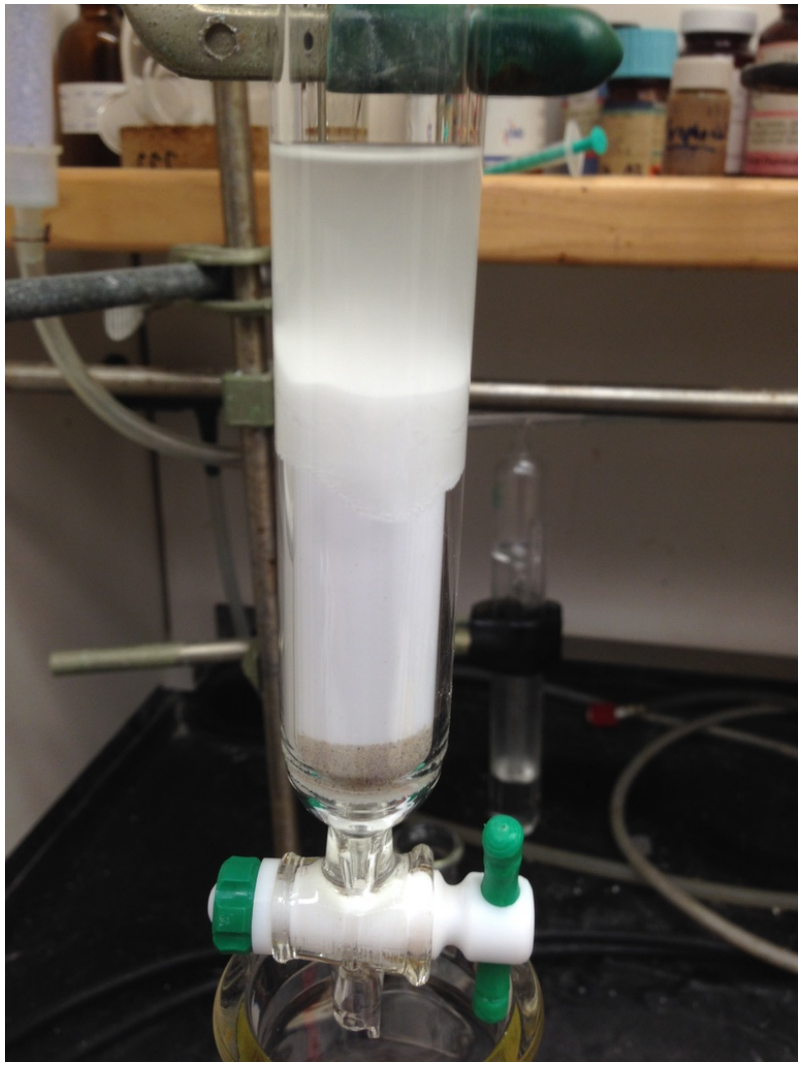
Packing the Column

- Add 1 cm of sand and level it



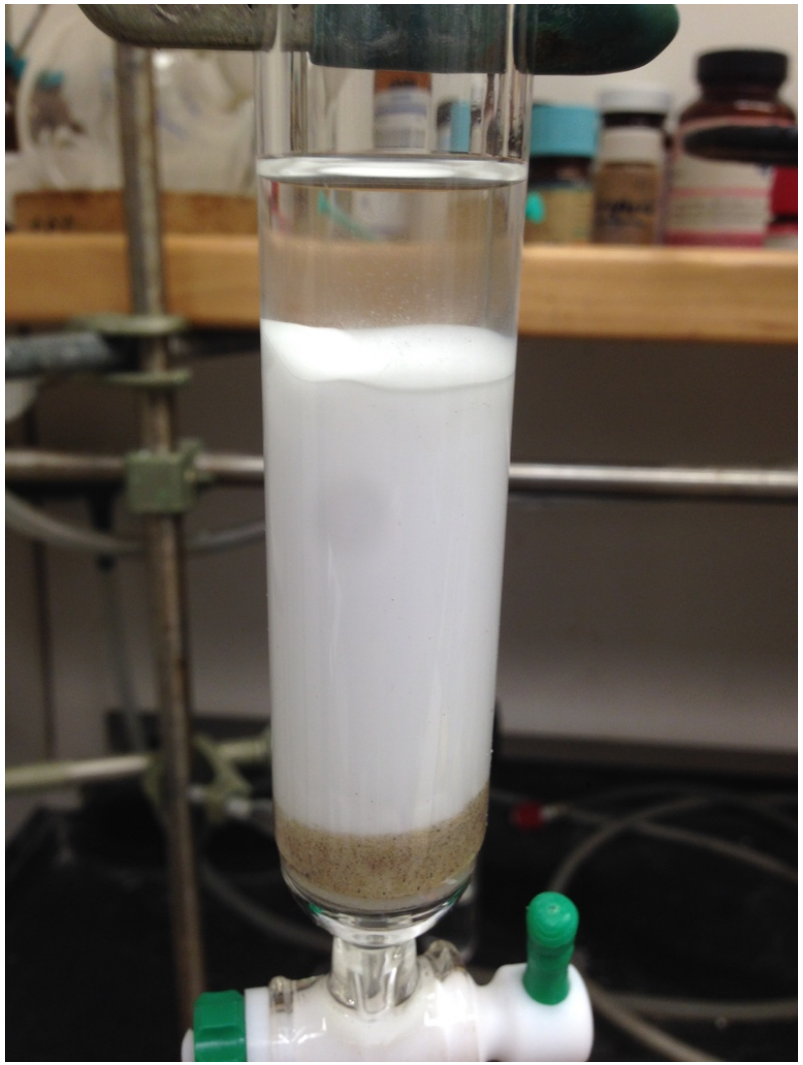
Packing the Column

- Add 2 – 4 in of the stationary phase
- Flush with HPLC-grade or distilled MeOH to pack
  - Too fine to pack dry



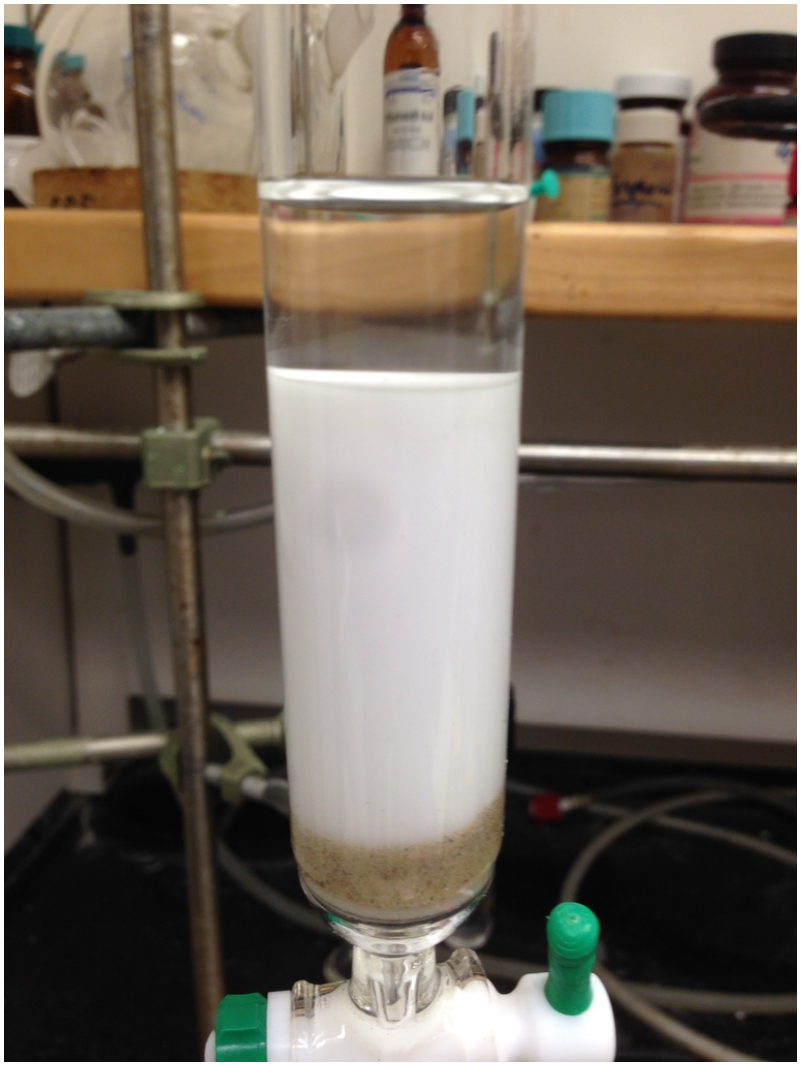
Packing the Column

- Leave a little MeOH to level the silica gel



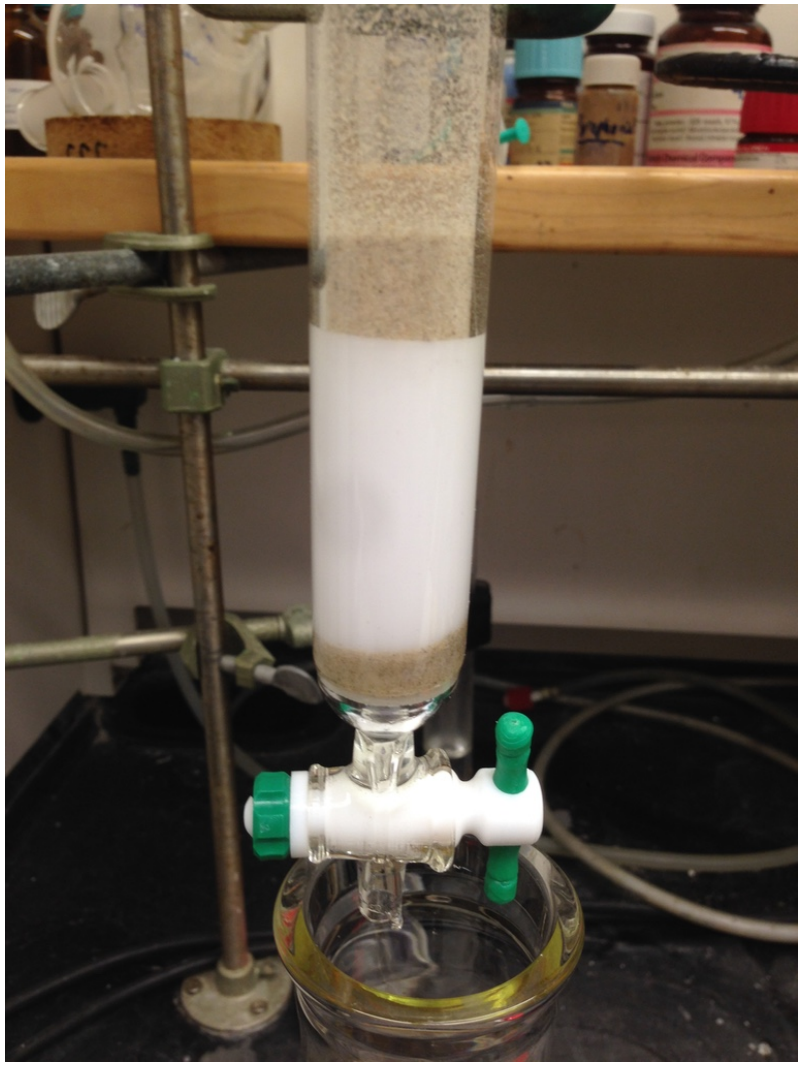
Packing the Column

- Fully packed and ready for last sand layer



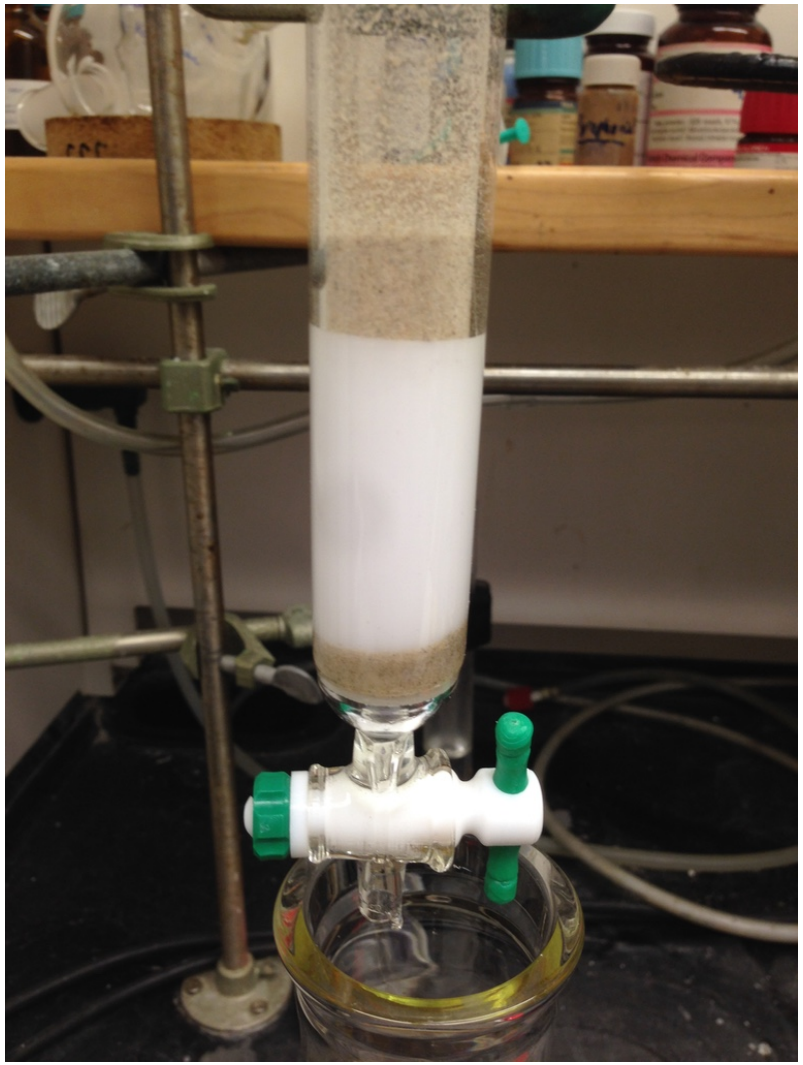
Packing the Column

- After lowering MeOH level, add 2 cm of sand



Packing the Column

- After lowering MeOH level, add 2 cm of sand
- Wash sand down and flush with solvent system you are going to use
- The column is ready for loading



Loading the Column

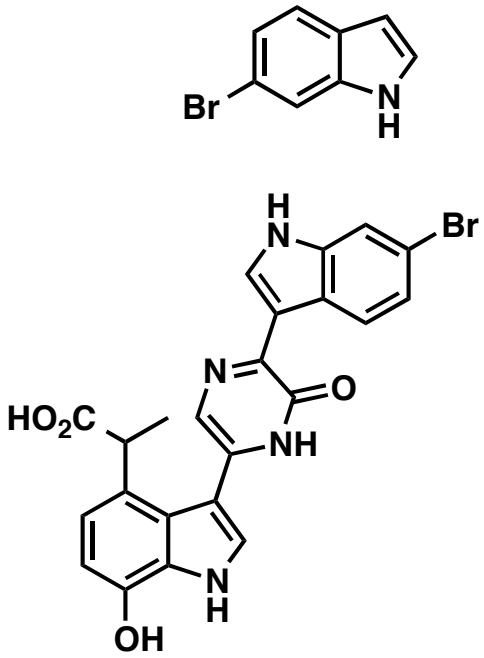
- Load crude material onto column using pure H_2O
- If insoluble, use solvent system you will be running
- Use sonicator to help with dissolution if solubility is low

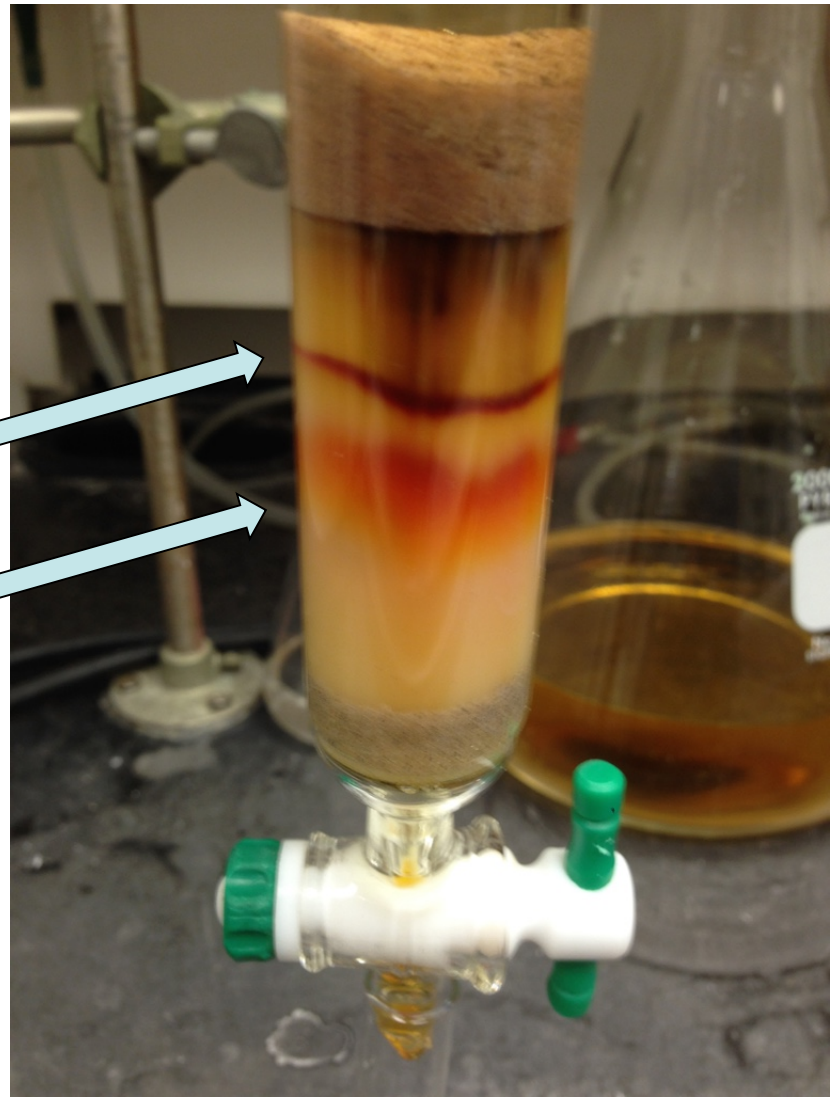
Running the Column

- When adding solvent, be careful not to disturb sand-stationary phase layer
- CAUTION – Generates much higher pressures than normal-phase flash chromatography
  - Use in hood with sash lowered while running, as always
- You can monitor fractions using NP TLC if R_f values are high enough (try 100% EtOAc w/0.5% AcOH

Running the Column

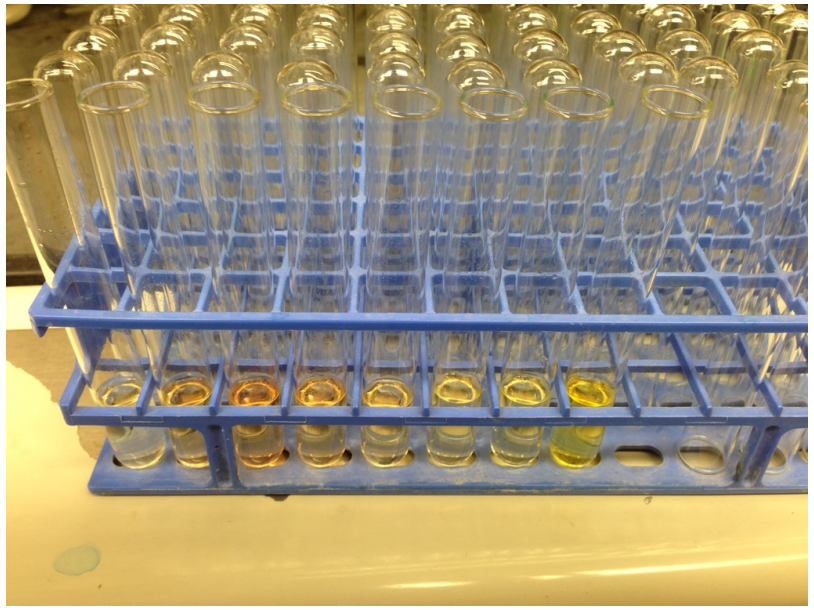
- Sometimes you can see the different products separating

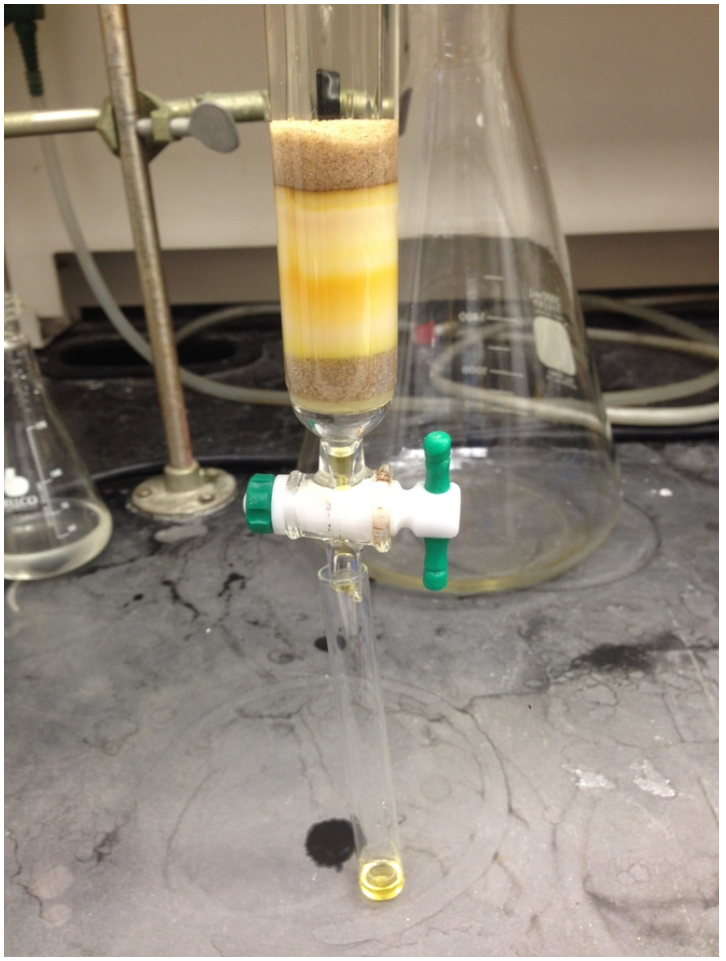




Collecting Fractions

- 13 mm i.d. column: 1-2 cm*
- 20 mm i.d.column: ⅓*
- 25 mm i.d. column: ½*

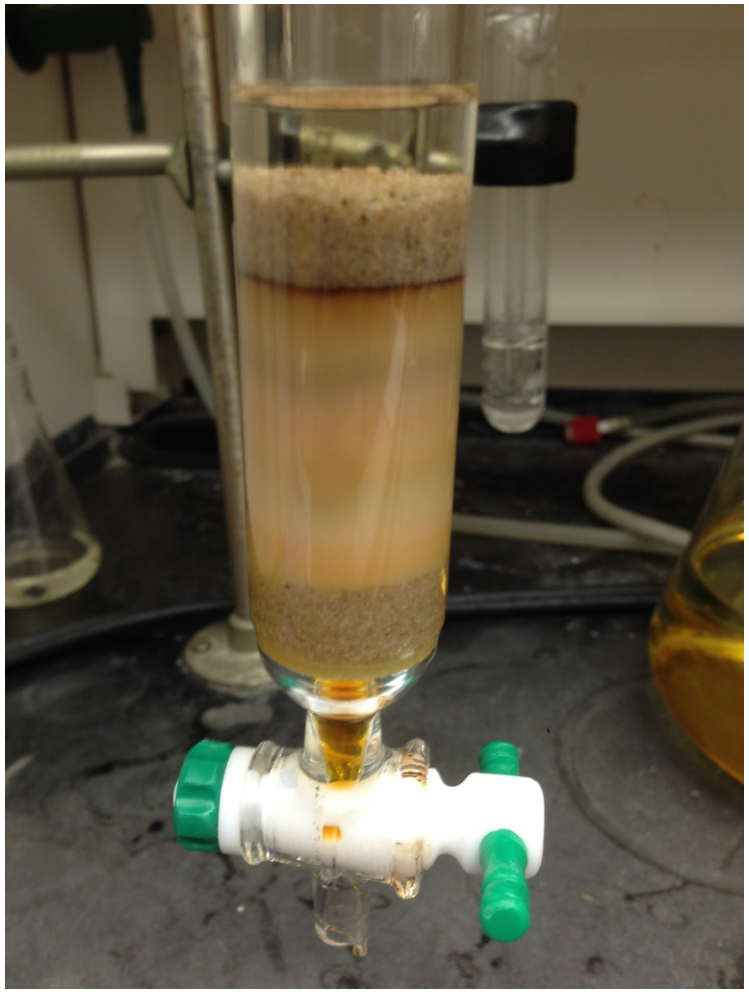




*13 x 100 mm test tubes

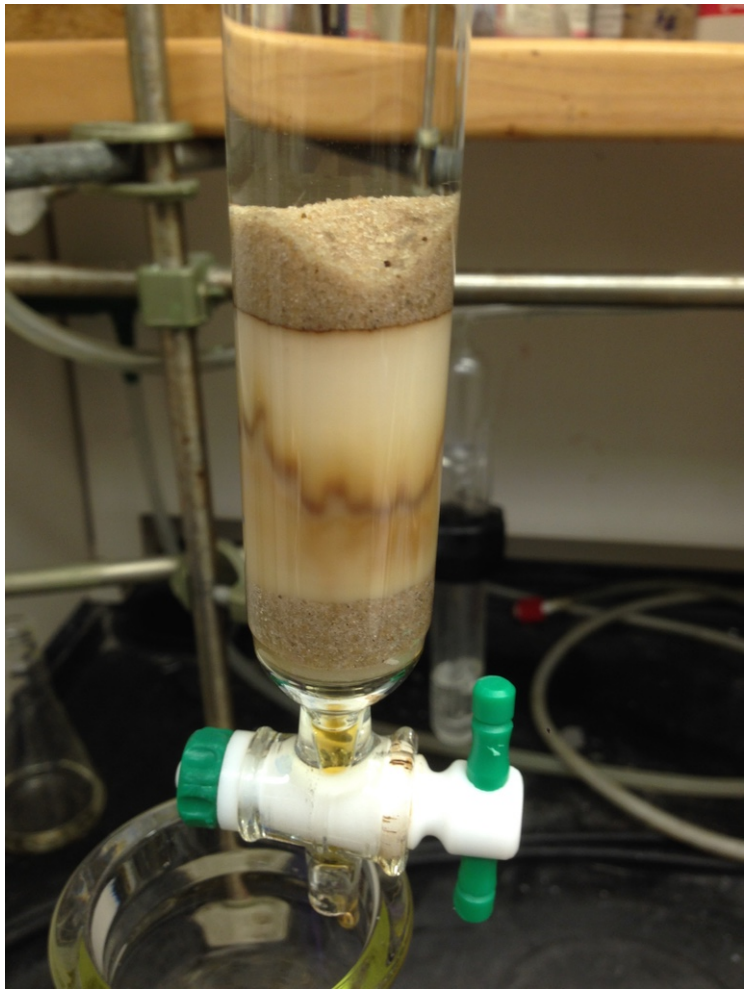
Cleaning the Column

- After the separation is complete, clean column for next separation



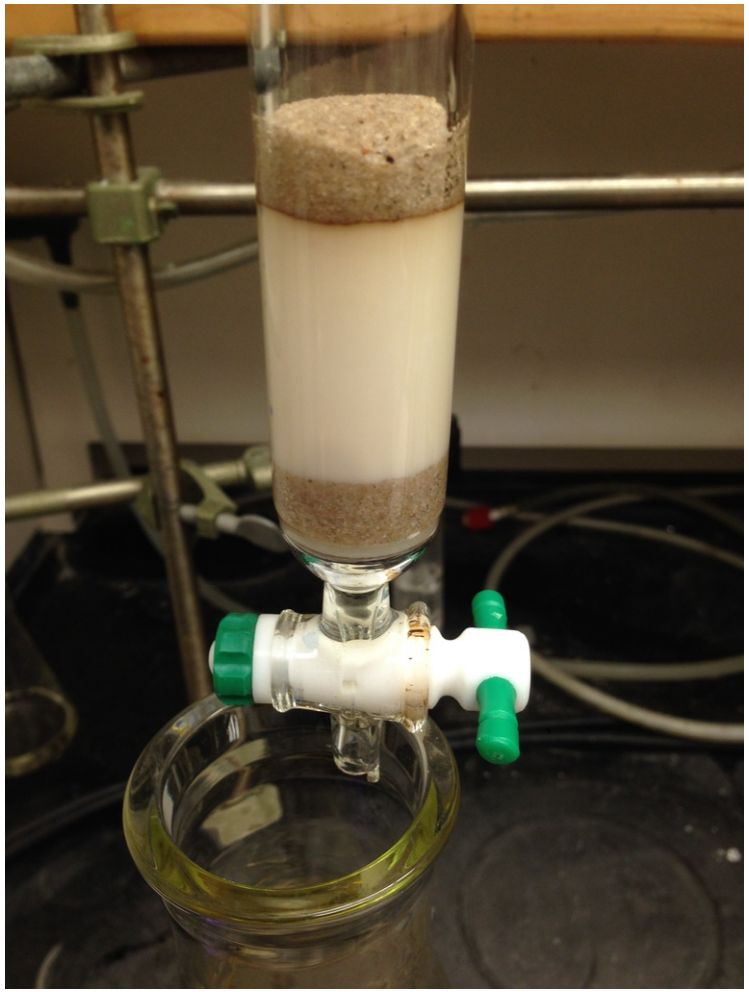
Cleaning the Column

- Flush with MeOH to wash the column



Cleaning the Column

- Ready for the next separation



The Solvents

NP Solvent	RP Equivalent
Hexanes	H_2O
EtOAc	MeOH
MeOH	MeCN

- Typically start with
  - 50:50 MeOH:H_2O w/ 0.1% TFA
  - 50:50 MeCN:H_2O w/ 0.1% TFA
- Optimize to set R_f = 0.3 using RP TLC