

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

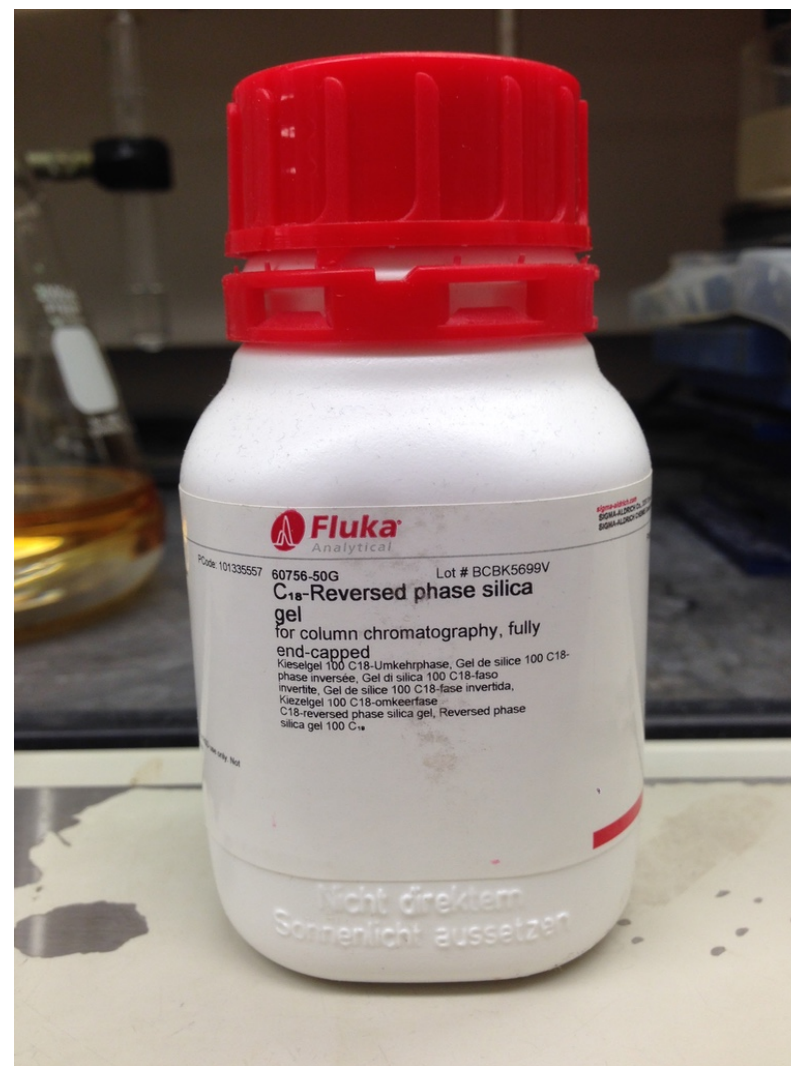
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# The Stationary Phase

- Fluka 60756-50G
- C<sub>18</sub>-Reversed phase silica gel
  - For column chromatography, fully end-capped
  - 15-25 um particle size
  - 100 Å pore size



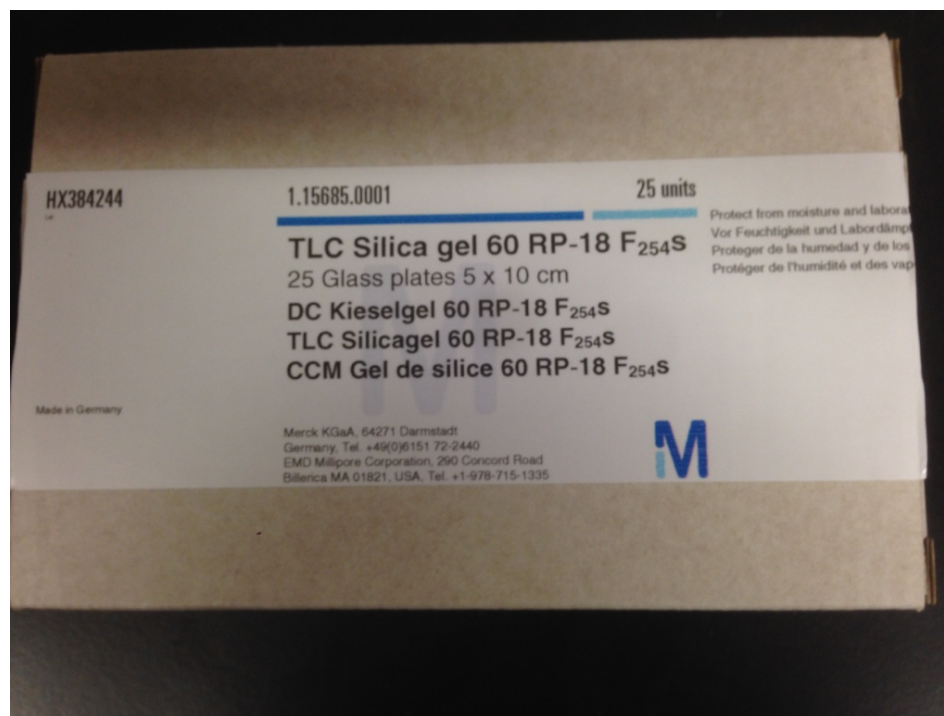
# Price Comparison

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- C18 Reversed-Phase  $\text{SiO}_2$  – Fluka  
(Sigma-Aldrich)
  - \$250 / 50 g
- Normal-Phase  $\text{SiO}_2$  – Grace DAVISIL  
(Fisher)
  - \$393 / 25 kg
    - \$0.79 / 50 g

# C18 RP TLC Plates

- EMD Millipore 15685
- TLC Silica gel 60 RP-18 F<sub>254</sub>S
- 25 count
  - 5 x 10 cm



# Comparing Price

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- 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

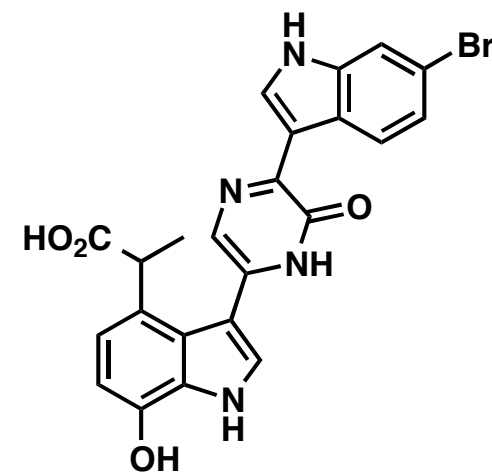
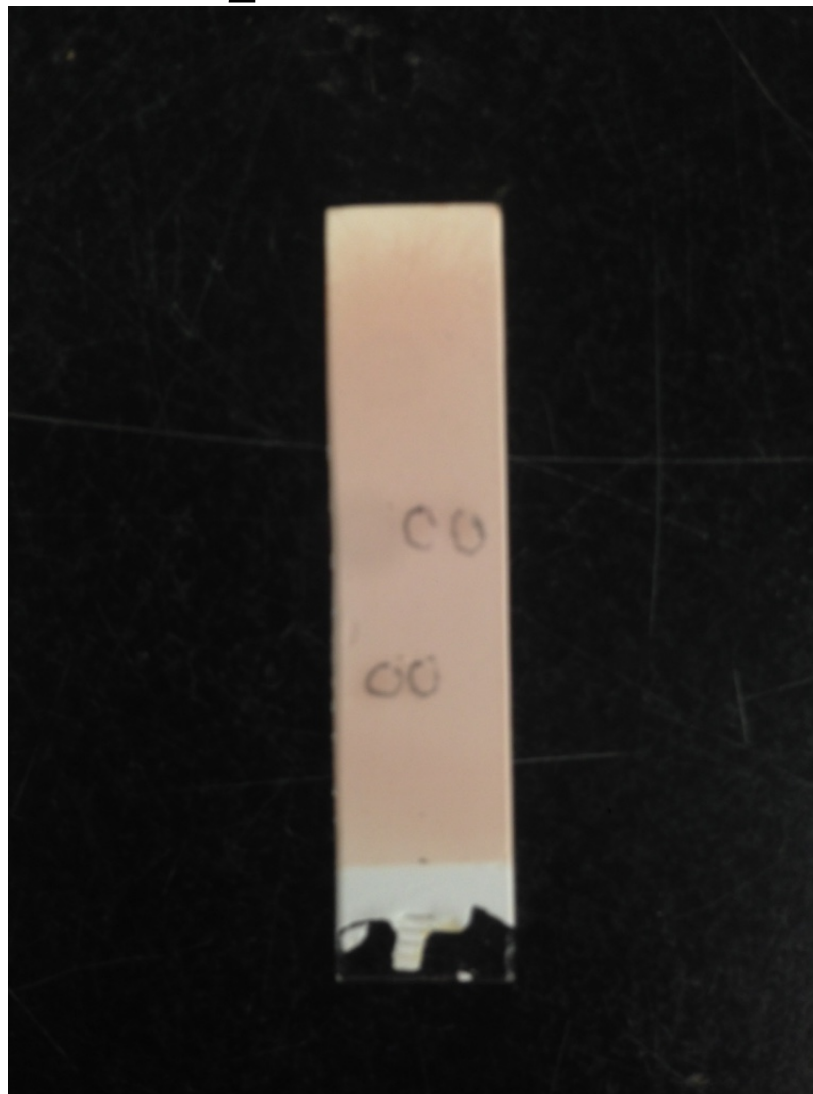
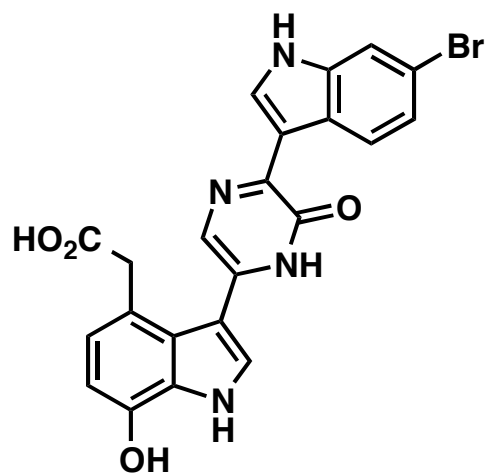
# TLC Plates

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



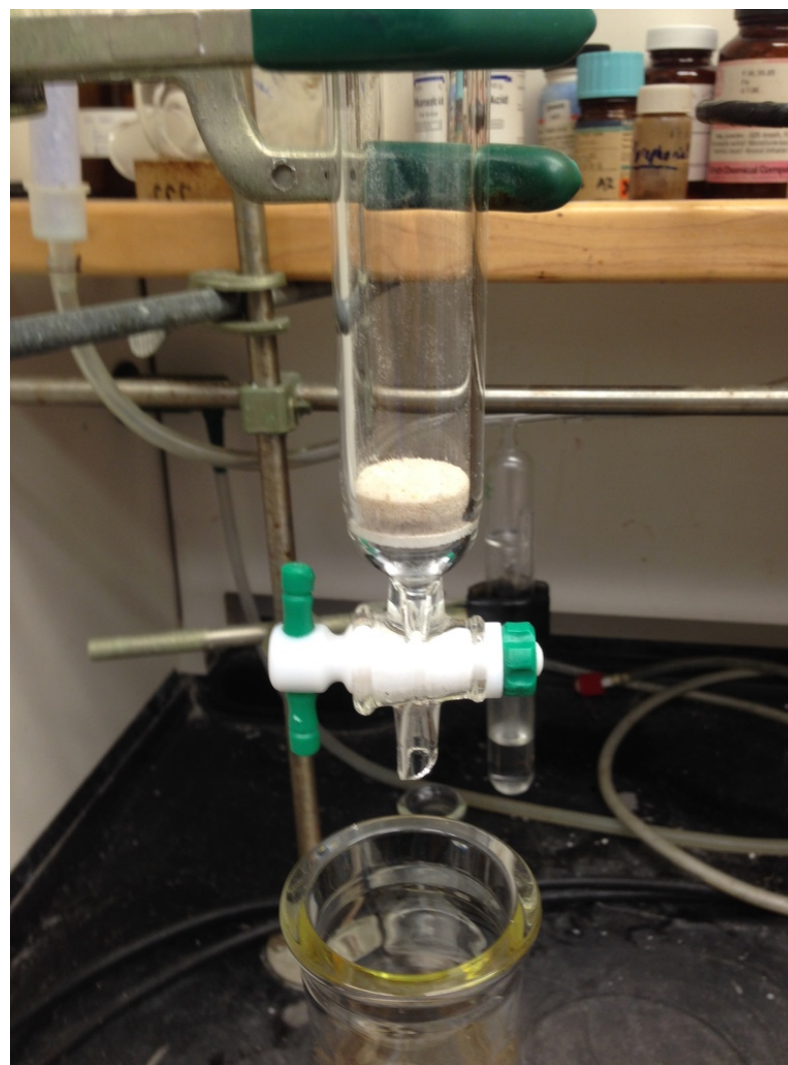
# Example of TLC

- 50:50 MeCN:H<sub>2</sub>O w/ 0.1% TFA



# Packing the Column

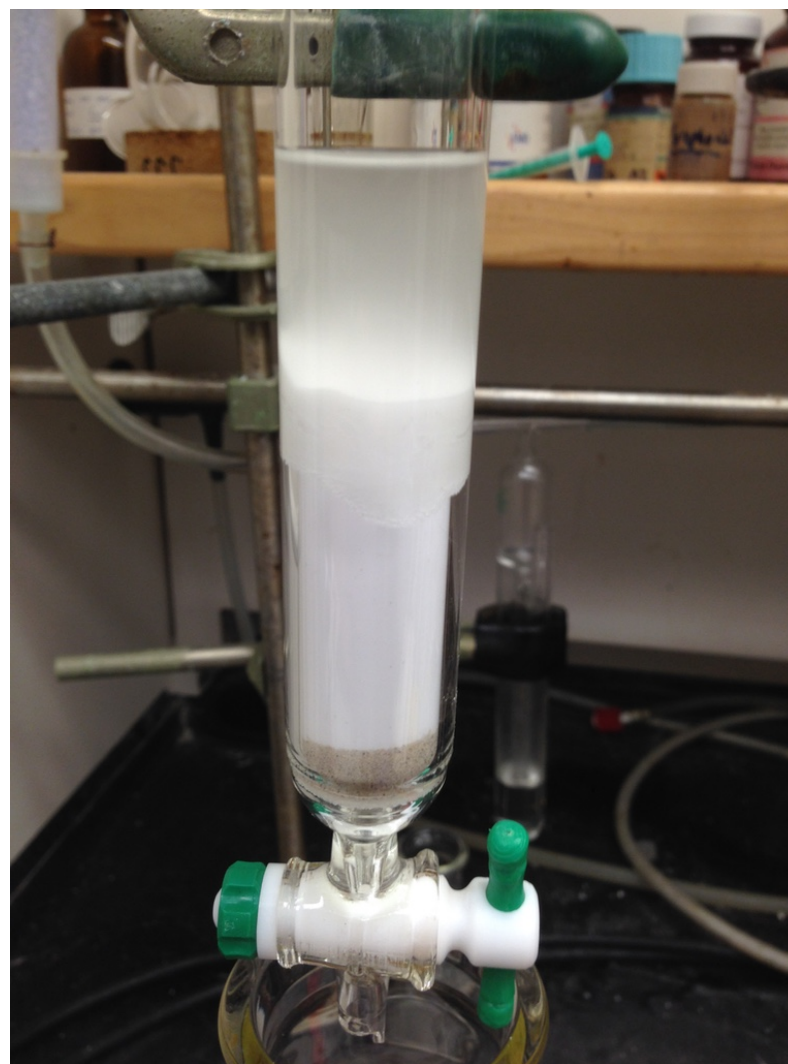
- Add 1 cm of sand and level it





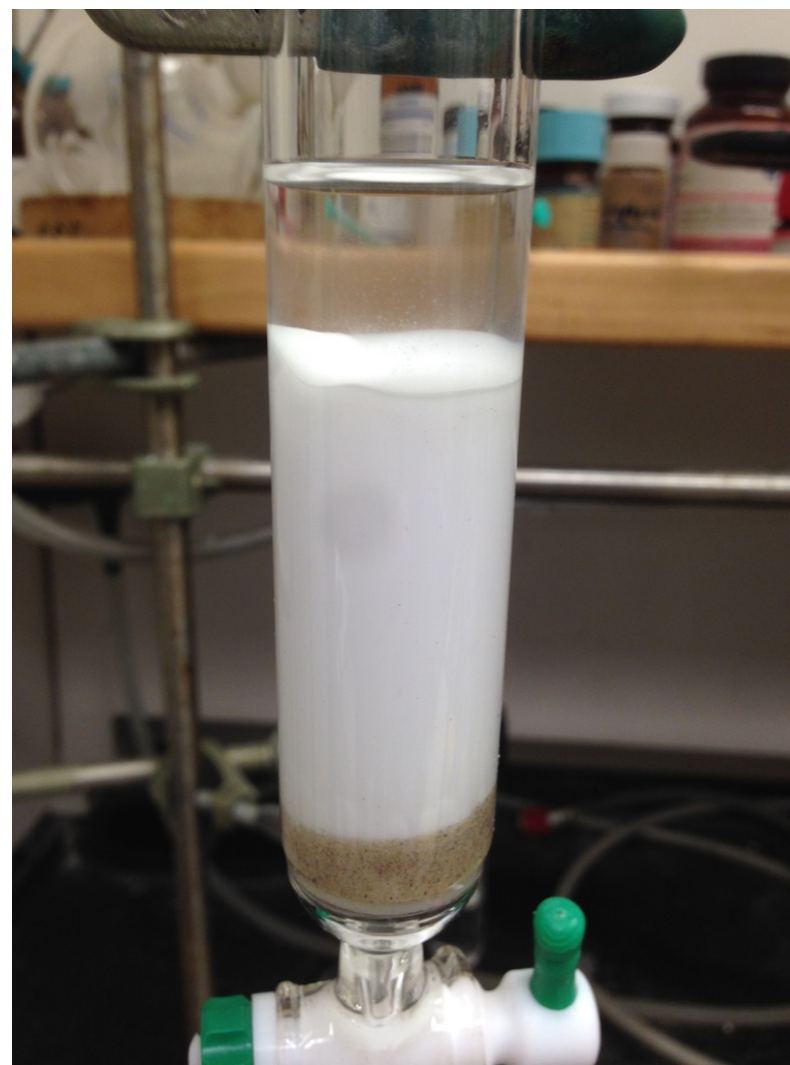
# Packing the Column

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



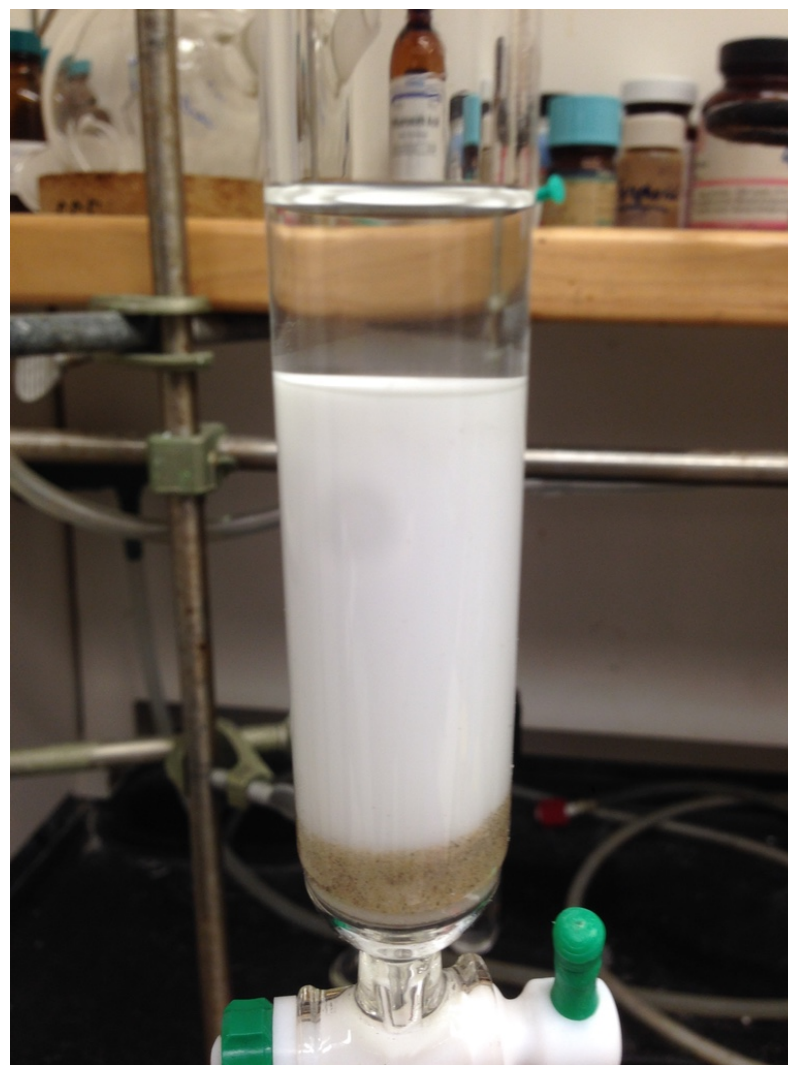
# Packing the Column

- Leave a little MeOH to level the silica gel



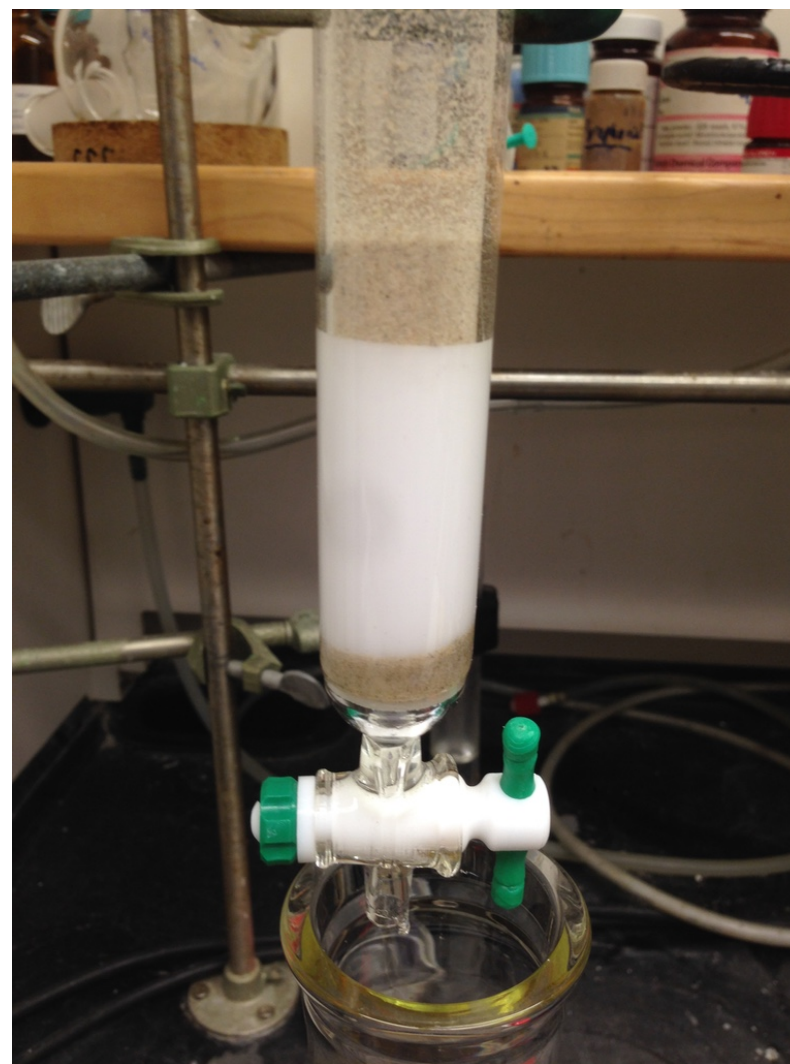
# Packing the Column

- Fully packed and ready for last sand layer



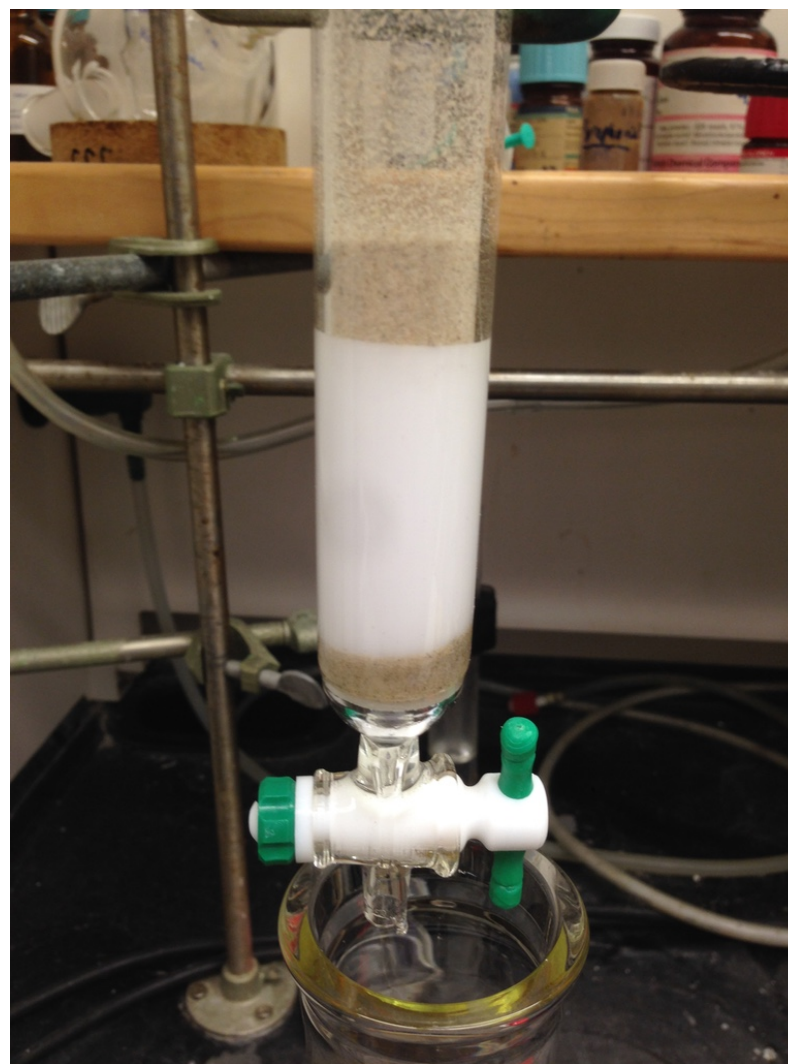
# Packing the Column

- 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

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- Load crude material onto column using pure H<sub>2</sub>O
- If insoluble, use solvent system you will be running
- Use sonicator to help with dissolution if solubility is low

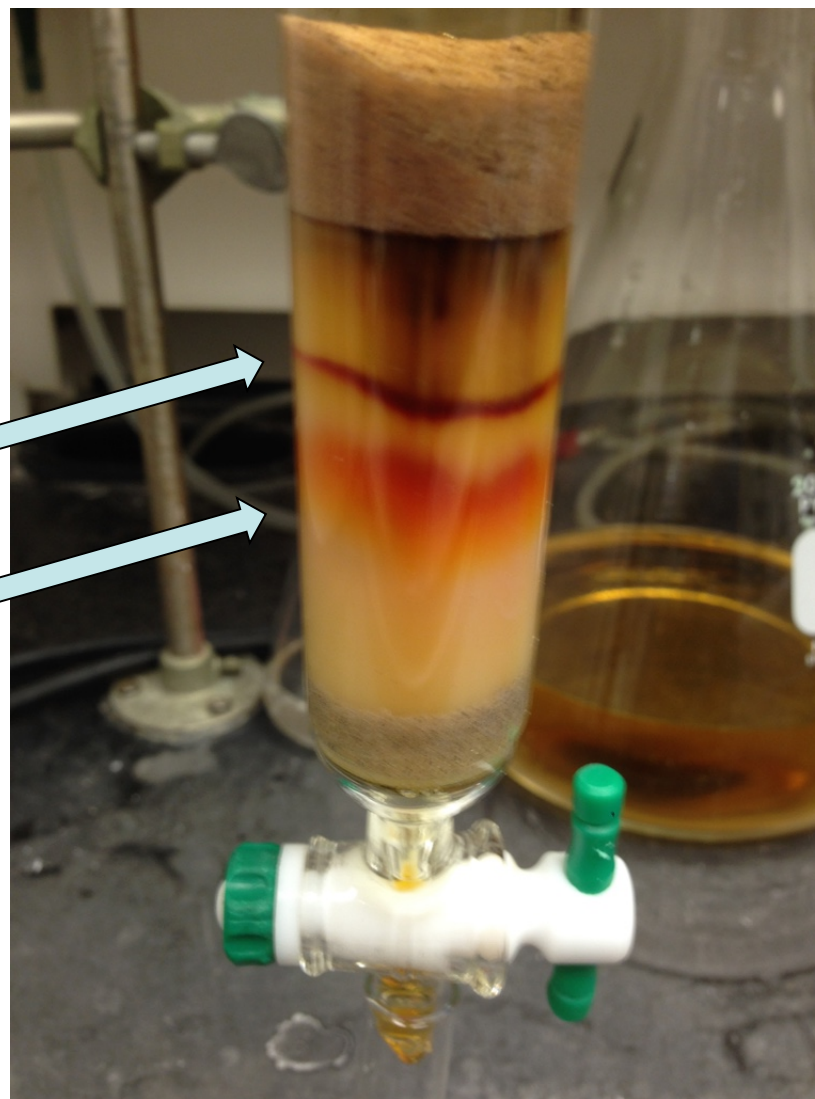
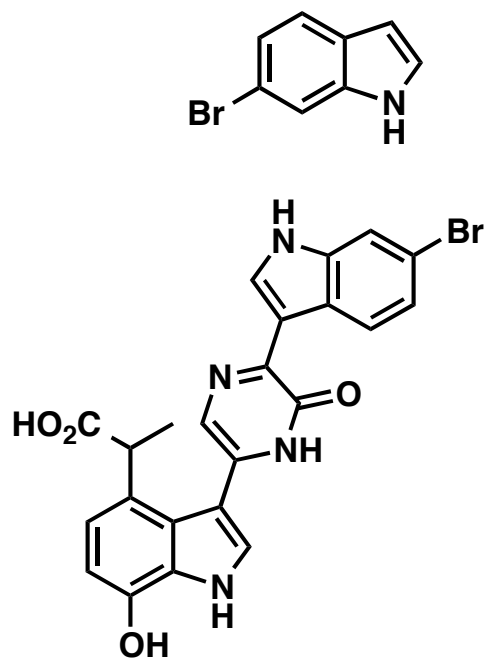
# Running the Column

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- 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

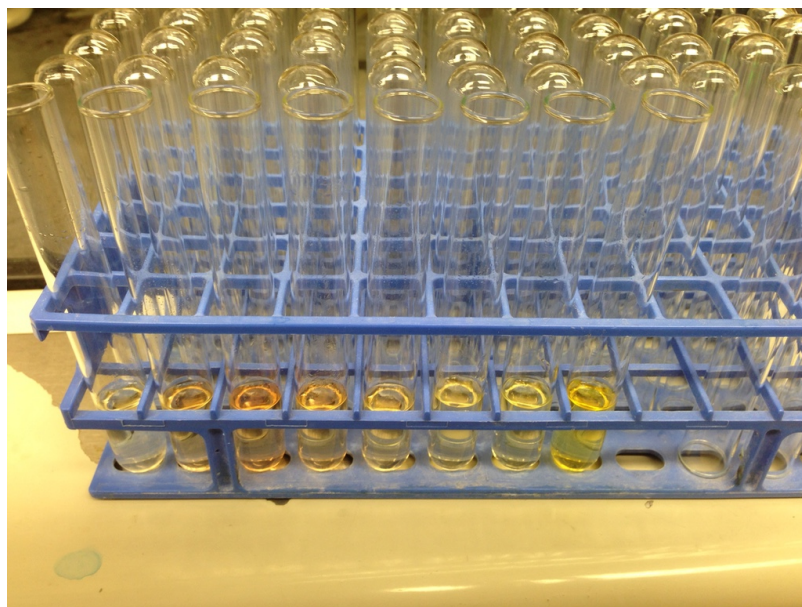
- Sometimes you can see the different products separating



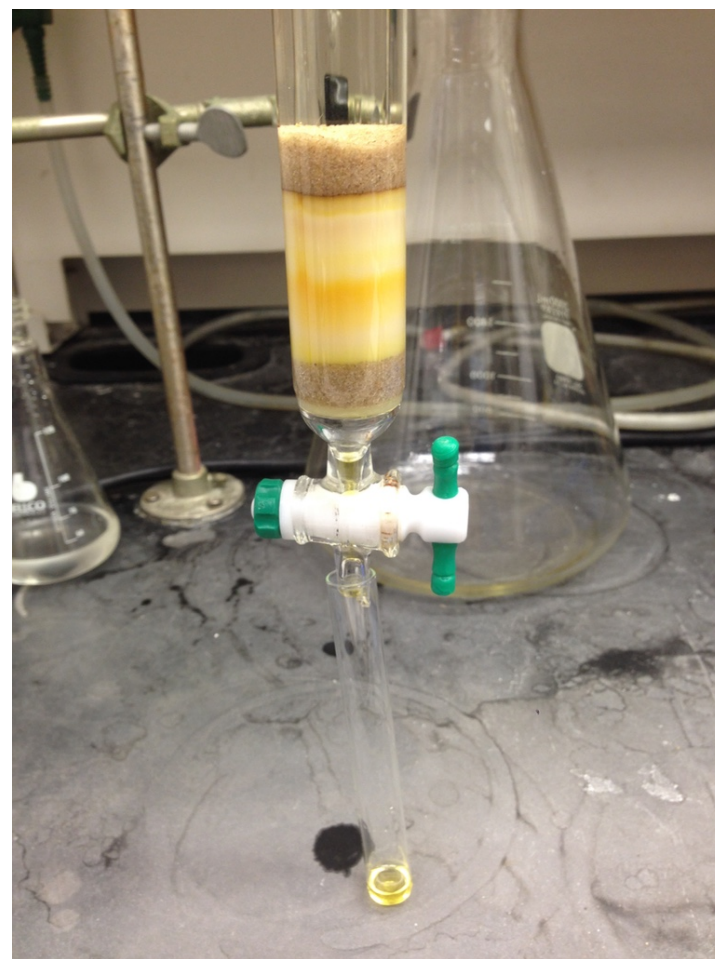


# Collecting Fractions

- 13 mm i.d. column: 1-2 cm\*
- 20 mm i.d. column:  $\frac{1}{3}$ \*
- 25 mm i.d. column:  $\frac{1}{2}$ \*

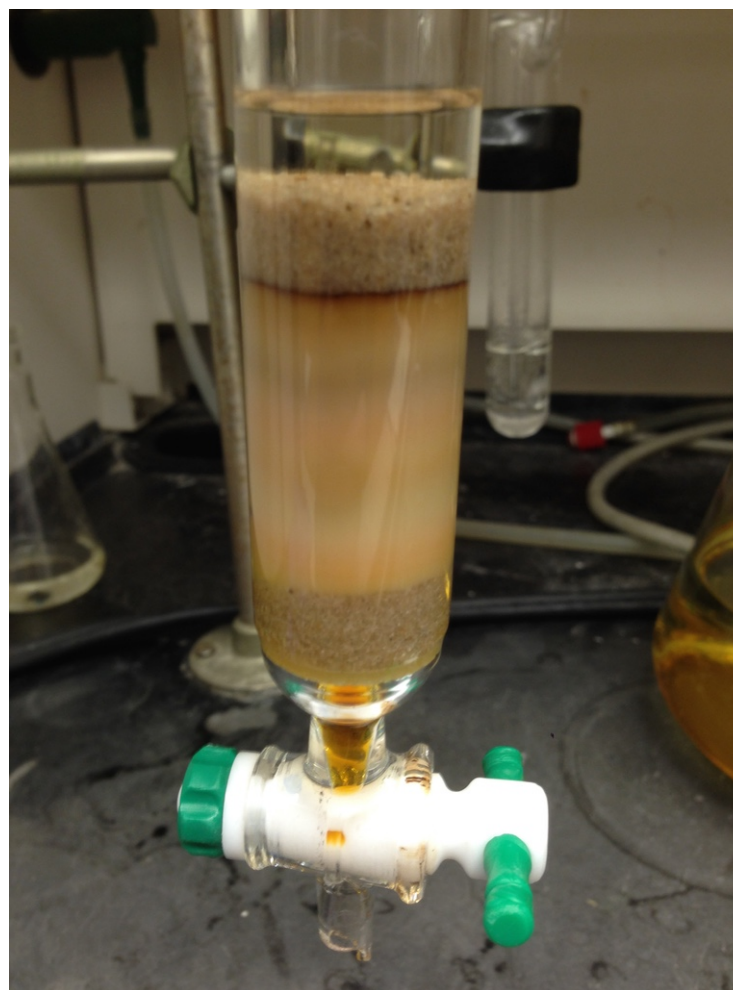


\*13 x 100 mm test tubes



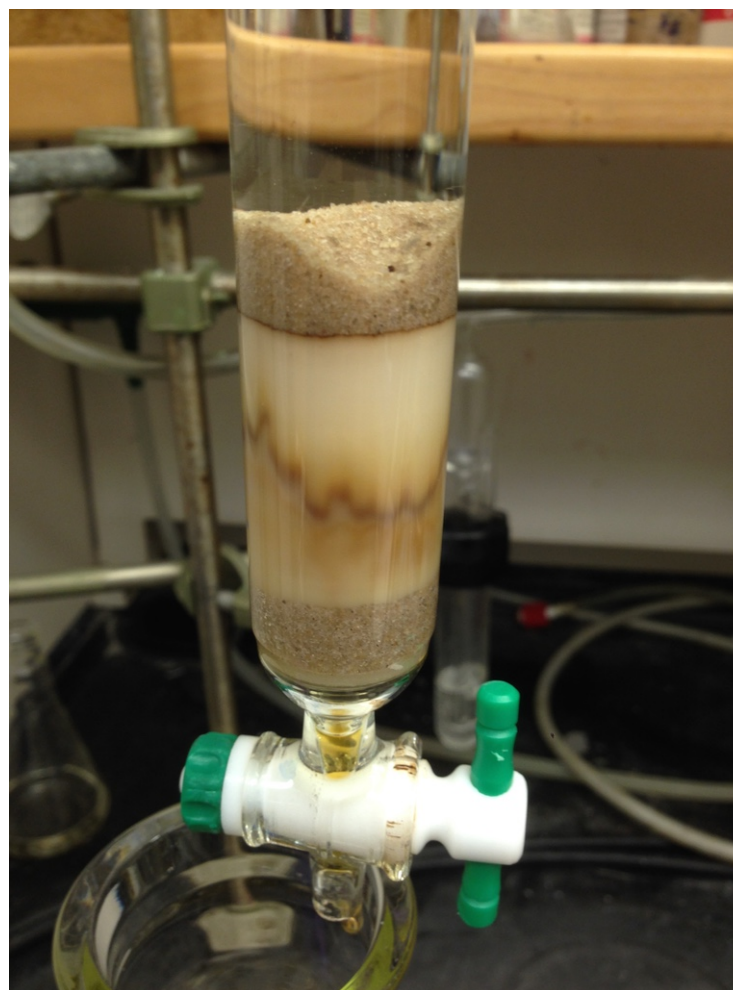
# Cleaning the Column

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



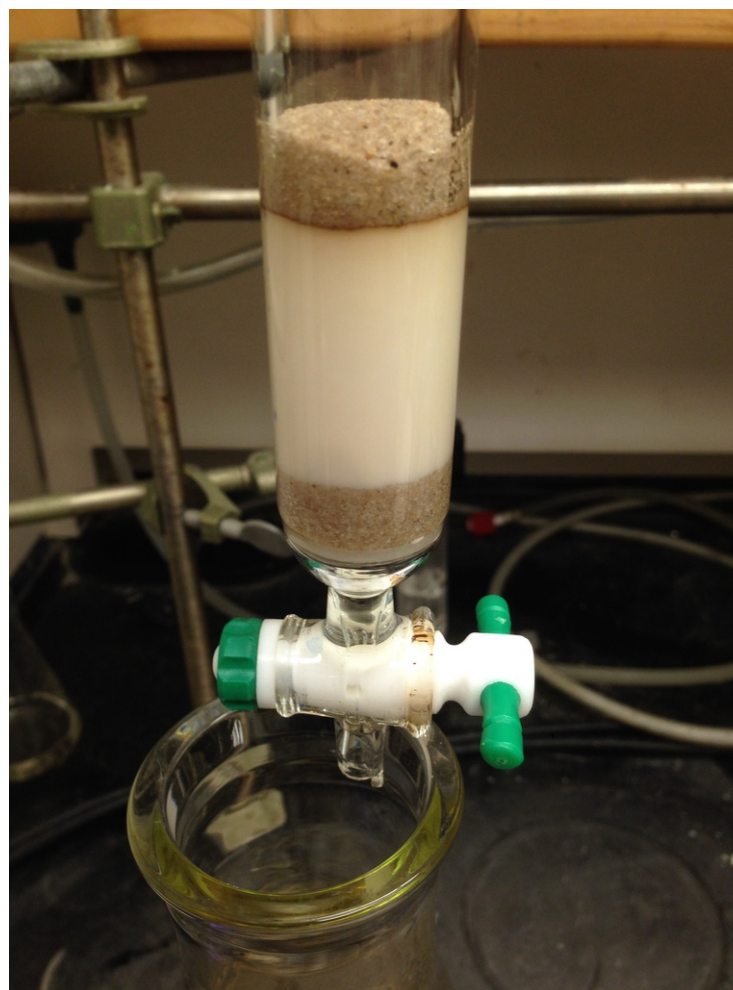
# Cleaning the Column

- Flush with MeOH to wash the column



# Cleaning the Column

- Ready for the next separation



# The Solvents

NP Solvent	RP Equivalent
Hexanes	H <sub>2</sub> O
EtOAc	MeOH
MeOH	MeCN

- Typically start with
  - 50:50 MeOH:H<sub>2</sub>O w/ 0.1% TFA
  - 50:50 MeCN:H<sub>2</sub>O w/ 0.1% TFA
- Optimize to set  $R_f = 0.3$  using RP TLC