***Quick Guide to Column Chromatography***

**Interpreting TLC Plates**

* Know how to calculate Rf



* A good gradient will start with a solvent system where the Rf of the top spot is ~0.3 and end with a solvent system where the Rf of the bottom spot is ~0.3.

**Solid and Mobile Phases**

* The solid phase of any chromatography system is the column. The mobile phase is the solvent.
* Make sure you select a solvent system that is appropriate for the column you are using.
* Start your gradient with a **low % of solvent B** and go up.

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| --- | --- | --- | --- |
| **Solid phase** | **Solvent A** | **Solvent B** | **Common moblie phases (A/B)** |
| Silica (normal phase) | Nonpolar | Polar | Hexanes/Ethyl acetate  DCM/Methanol |
| C18 (reverse phase) | Polar | Nonpolar | Water/Acetonitrile  Water/Methanol |

* For **basic** (i.e. nitrogen containing) compounds, it is sometimes useful or necessary to add a small amount of triethylamine or pyridine to the solvent mixture (about 0.1%) when running a normal phase column.
* For **acidic** compounds, a small amount of acetic acid is sometimes useful.

**Loading Compounds**

* Dry loading usually works best for most compounds.
* Wet loading can work well if your compound is fully soluble in DCM and doesn’t streakor travel too fast through the column. An easy way to check if wet loading with DCM is suitable, is to do a TLC in CH2Cl2 and check the Rf of your compound.

**Biotage Maintenance**

* If changing solvent bottles, make sure you change back at the end of the run to the standard setup:

**S1** = Hexanes

**S2** = Ethyl acetate

**S3** = Dichloromethane

**S4** = Methanol

* Switch to full solvent bottles when levels are low. Bottles with low levels may be left in the hood and used to top up partially used bottles, then discarded promptly.
* Clean and return test tube racks in a timely manner.
* The Biotage hood is a **glove-free area**.