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General Guidelines for prospective HPLC users

Before you start

1) Wear safety glasses and appropriate lab attire.

2) Organic solvents are used for this instrument (usually hexanes, isopropanol, ethyl acetate, and dichloromethane). Make sure you read the MSDS of these solvents before starting experiment.

What do I need to do to get started?

1) The HPLC does not operate by a sample submission service. This means that if you want to run a sample, then you must be fully trained on the instrument and run the sample yourself. Training requires at least 1 hour of personal instruction from the HPLC manager. Therefore, request for training should be made at least 3 days in advance.
2) All samples must be a) soluble in the elution solvent that you will use b) previously passed though a column or short plug of silica. *This means that crude reaction mixtures are not acceptable for HPLC runs. Extremely polar or insoluble impurities will become trapped on the column and cause blockage.*
3) The compound of interest must have some absorbance in the UV-range to be noticed by the detector.
4) The **achiral** prep scale and analytical columns use the four main solvents used in regular flash chromatography on silica gel (CH_2Cl_2, EtOAc, MeOH, and hexanes). Other solvents may only be used if you find precedence (i.e. manufacturer recommendations) that the solvent is acceptable for the column to be used. This will be the responsibility of the user, not the HPLC manager.
5) The **chiral** analytical columns may only use **isopropanol** and **hexanes** as the elution solvent in the range of 0% to 15% isopropanol in hexanes. *Use of any other solvents (even in residual amounts) will cause immediate and irreversible destruction of the column*. The samples must be dissolved in isopropanol and hexanes and filtered though a syringe filter before applied to the chiral column.

Why would I want to use the **achiral** preperatory HPLC?

The best use of the prep scale HPLC is to isolate small amounts of analytically pure compound for characterization (NMR, microanalysis, etc.).

You should not rely on the prep scale HPLC for routine purification of synthesis materials on a large scale. The ideal scale for prep scale experiments is **5-50 mg.** Larger sample loading may be possible with easily separated mixtures, but in this case it is usually more convenient for you to simply run a flash column in your hood.

Why would I want to use the **achiral** analytical HPLC?

The best use of the achiral analytical column is for reaction monitoring or to test for product purity.

It is not suggested to use the analytical column to test the separation with a particular method before trying the prep scale column. There is actually only a loose correlation between peak resolutions between the analytical and prep columns. Because samples are recovered in fractions after the prep column, it is far easier to rotovap the fractions down and try the separation again if the first method didn't work.

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Why would I want to use the **chiral** analytical HPLC?

The chiral HPLC columns are used to determine the enantiomeric (or diastereomeric) ratios of chiral samples.