Agilent Chiral Chromatograph Training Guide

Introduction

The chiral chromatograph separates enantiomers using special columns containing a stationary phase that consists of a single enantiomer of a chiral compound. Because achieving a successful separation can be a bit of a guessing game, the chromatograph is equipped with four different chiral columns, allowing users to quickly try a wide variety of methods. After separation in the column, the compounds pass through a UV-Vis absorbance detector.

Stuff to Buy

Vendor	Part Number	Description
VWR	66009-874	Sample vials with caps and septa
VWR	10754-820	1 L glass storage bottles

Appropriate Samples and Sample Prep

Samples must be dissolved in hexane, isopropyl alcohol, or a mixture thereof. **Samples must not contain other organic solvents in significant quantities.** Basic or acidic samples require modifiers be added to the mobile phase according to the following table:

Basic samples require basic modifiers	Acidic samples require acidic modifiers
Diethylamine	Trifluoro acetic acid
Butyl amine (for primary amines)	Acetic acid
Ethanol amine (for primary amino alcohols)	Formic acid
Concentration: 0.1% v/v	Concentration: 0.1% v/v

Approximately 1ml of sample is required for analysis. Analyte concentration typically ranges from 0.01-1 mg/ml, although detection limits vary with analyte absorbtivity and background absorbance from either the mobile phase or other components of the sample. Samples that may contain particulates must be passed through a filter with pore size no larger than 0.5 μm prior to analysis.

The HPLC uses a UV-Vis absorbance detector with a wavelength range of 190-700nm. Analytes must absorb in this wavelength range to be detectable. Additionally, solvents and additives in the mobile phase must not absorb in the same region as your analyte. If possible, it is best to look up or collect a UV-Vis spectrum of your analyte in order to identify appropriate wavelengths for detection.

Getting Started

1. Examine the solvent bottles to ensure that there is sufficient solvent to perform your analysis. The instrument uses approximately 1ml/min of solvent while running. Contact RIC staff if you need more solvent. The contents of the four solvent bottles are as follows:

   A1: 99.5% hexane, 0.5% isopropyl alcohol
   B1: Isopropyl alcohol
   A2: Hexane
   B2: 99% hexane, 1% isopropyl alcohol

   DO NOT USE DIFFERENT SOLVENTS WITHOUT PRIOR APPROVAL FROM RIC STAFF! MANY SOLVENTS WILL DESTROY THE CHIRAL COLUMNS EVEN IN SMALL AMOUNTS! THE TOTAL VALUE OF THE CHIRAL COUMNS IS ALMOST $10,000 SO THIS IS A VERY EXPENSIVE MISTAKE TO MAKE!

2. Examine the waste container in front of the instrument and make sure it is not full and will overflow during your experiment. If the was container is full, contact RIC staff to have it emptied.
3. Turn on the chromatograph and the column thermostat using the buttons on the front.
4. Log in to the PC and start the *Control Panel* software using the desktop icon. Click *Launch* under *Start Instrument*, then click *New method from instrument*.
5. In the lower left corner click *Method and Run Control*
6. Open the front cover of the chromatograph by pushing in the two semicircular buttons on the sides (see pic) and tilting the cover downwards. Open the purge valve by turning it half a turn counter clockwise.
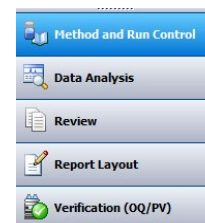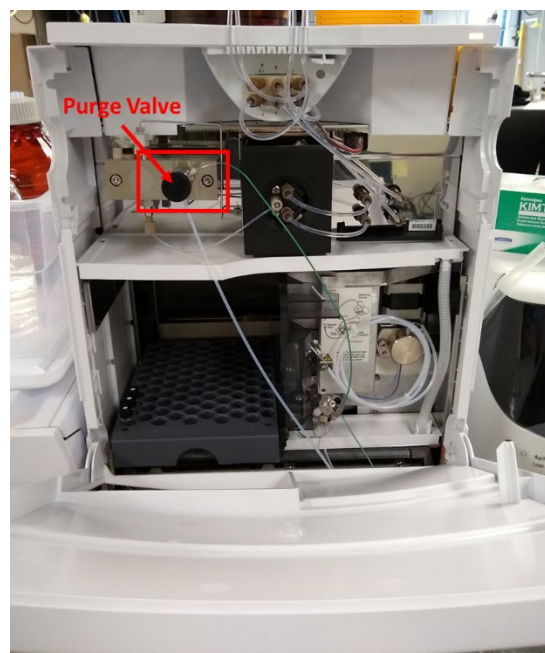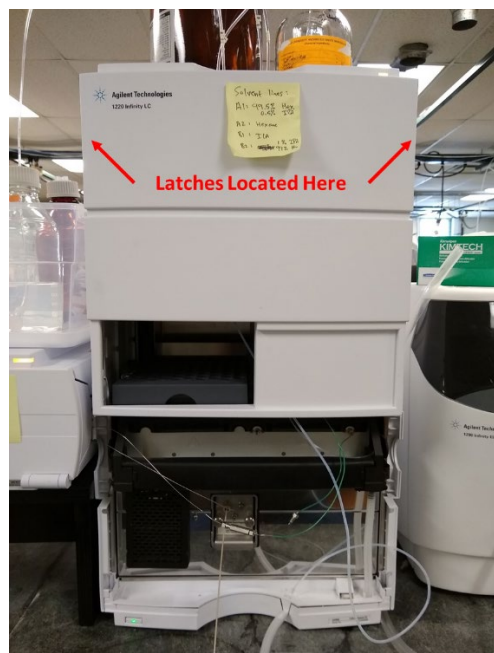
7. On the menu bar click *Instrument* → *System On* to start the pump
8. On the menu bar click *Instrument* → *Set up Instrument Method*
9. On the *Grad Pump* tab, change the flow rate to 1ml/min and change the solvent composition to flow 100% from a single bottle that you wish to use and click *Apply*. While the solvent is flowing, check the transfer lines for air bubbles. Allow the solvent to flow for at least 1 min once air bubbles are no longer observed.
10. Repeat the previous step for all other solvents you will be using, then click *Ok* in the *Run Setup* window.
11. On the menu bar click *Instrument* → *More Grad. Pump* → *Pump Power-Switch Off* to stop the pump. Close the purge valve by turning clockwise.
12. On the menu bar click *Instrument* → *More VWD* → *UV Lamp – Switch On*. The instrument is now ready for use.

Software Overview:

In the lower left corner you will find a menu that allows you to select different sections of the software. Each section has its own set of tabs near the top of the screen that allow access to different functions within the section.

1. *Method and Run Control* is for data acquisition. Here you can control the instrument and set up methods and sequences.
2. *Data Analysis* allows you to view previously acquired data, perform background subtractions, integrate peaks, etc. You can also generate pdf reports of your data and export csv files to open in Excel or other software.
3. *Report Layout* allows you to create custom reports to present your data in pdf format.
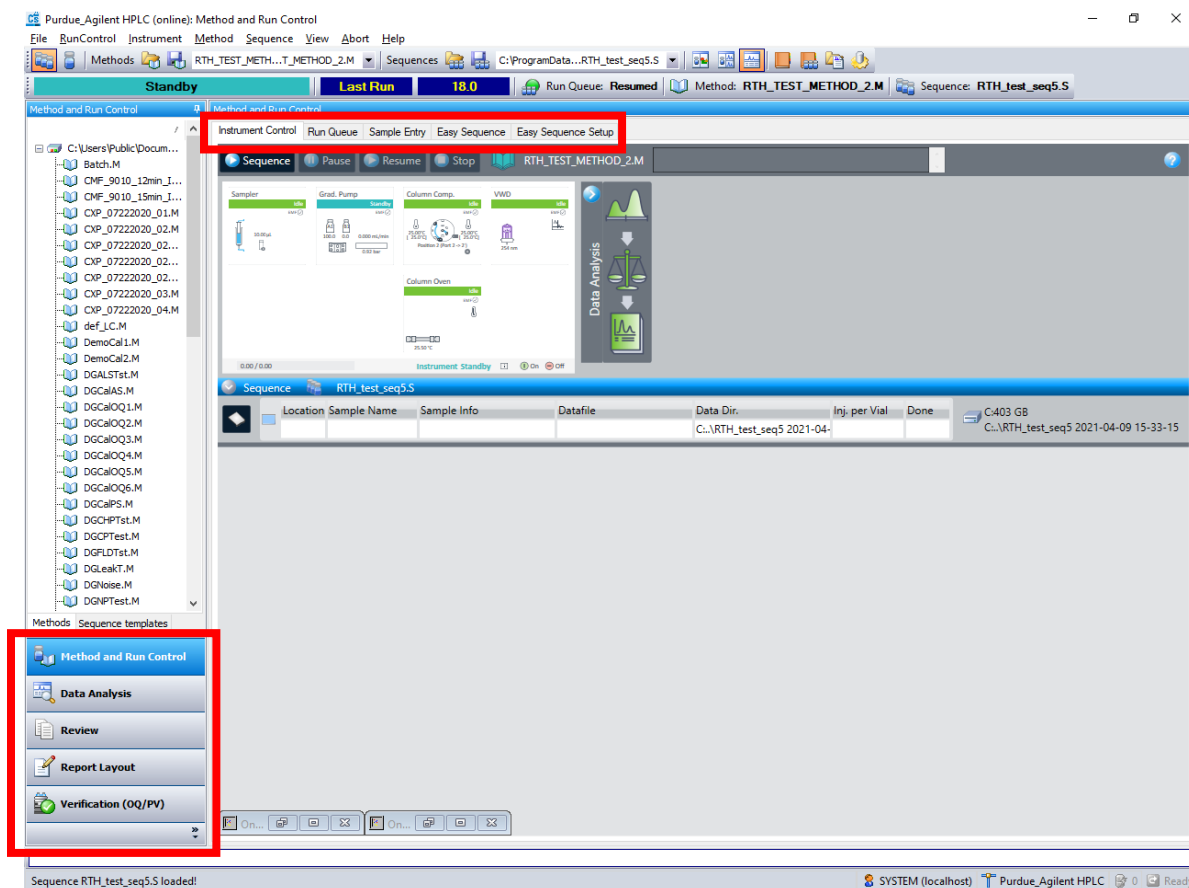
Creating a Method

The method contains all of the settings (gradient, flow rate, injection volume, detection wavelength, etc.) that the chromatograph will use when analyzing your samples. If you need a method to start from you can open

C:\Users\Public\Documents\ChemStation\1\Methods\RTH_example_method.M

1. On the menu bar click *Instrument* → *Set up Instrument Method*
    a. On the *Sampler* tab:
        i. Enter your desired injection volume
    b. On *Grad. Pump* tab:
        i. Set up your initial conditions under *Flow* and *Solvents*
        ii. Set up your gradient under the *Timetable*. Clear the timetable if you are doing an isochratic run.
        iii. If doing gradient elution, enter at least 8 min under *Posttime* for column equilibration between runs.
        iv. Under *Stoptime* enter the total time for one analysis (excluding posttime).
        v. You can use the *Timetable Graph* to verify that solvent conditions will change as you expect during the run.
   c. On *Column Comp.* tab:

      i. Under *Temperature,* in the left column, enter the desired column temperature. **DO NOT SET THE COLUMN TEMPERATURE ABOVE 40°C OR COLUMN DAMAGE MAY RESULT!**

      ii. Under *Valve Position/Column,* select the desired column. The choices are as follows:

        Position 1: Chiralcel OD-H 4.6mm x 250mm, 5µm particle size
        Position 2: Chiralpak AD-H 4.6mm x 250mm, 5µm particle size
        Position 3: Chiralpak IB-3 4.6mm x 250mm, 3µm particle size
   Position 4: Chiralcel OJ-H 4.6mm x 250mm, 5µm particle size

d. On *VWD* tab:
    i. Under *Signal* check *Acquire* and type in the wavelength. Under *Peakwidth* select the expected width of your chromatographic peaks.

e. Click *Apply* then *Ok*

f. Save your method by clicking *Method → Save Method As* on the menu bar

g. On the menu bar click *Instrument → More Grad. Pump → Pump Power – Switch On* to turn the pump on and begin equilibrating the column at your initial conditions
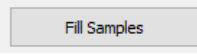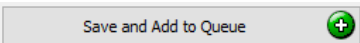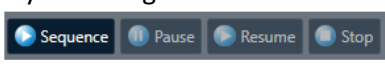

Creating and Running a Sequence

The sequence contains information about your samples and the associated method files that will be used to analyze them.
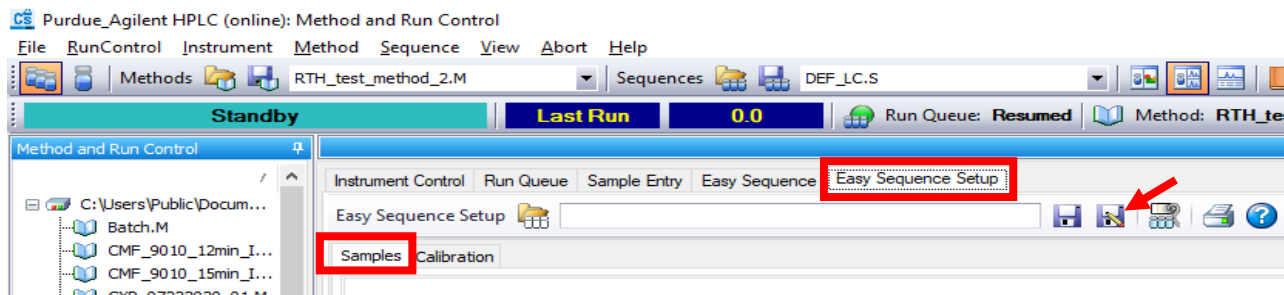
1. On the *Easy Sequence Setup* tab → *Samples* tab, select your method and enter the estimated cycle time (run time plus posttime).

a. Enter *Number of Samples*, *Starting Vial Location*, and *Sample Name*

b. Under *Data Location*, browse to your data directory. Data subdirectories should be created in C:\Users\Public\Public Documents\ChemStation\1\Data\

c. You do not need to enter a data file name the system will come up with something based on your sample names

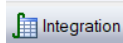d. Save your sequence setup by clicking *Save Sequence Setup As*
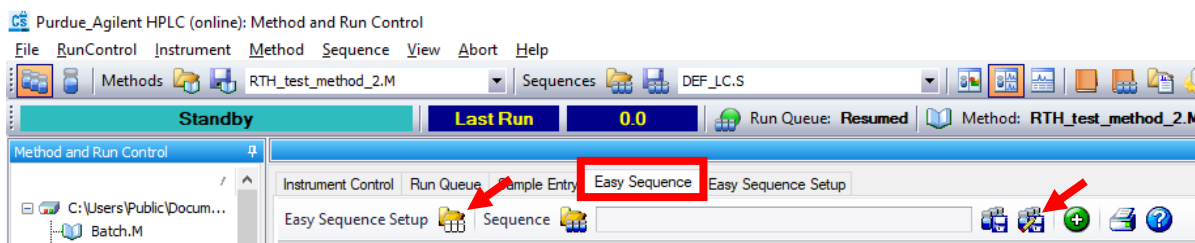
2. On the *Easy Sequence* tab, load your easy sequence setup by clicking
    a. Click [Fill Samples]

    b. Load your sample vials into the appropriate positions in the sample tray.
    c. Click to start your sequence [Save and Add to Queue ⊕]
    d. If the sequence does not start, you may need click on the *Run Queue* tab and start or unpause the queue, or you may need to go to the *Instrument Control* tab and start or unpause the sequence using [▶ Sequence ⏸ Pause ▶ Resume ⏹ Stop]
    e. If the instrument is not in a *Standby* or *Ready* state, as indicated in the upper left corner, then the instrument is not yet ready to run. Reasons for this include temperature setpoints or pressure stability requirements not being met, or UV lamp not on. To find out why the instrument is not ready, click on the *Instrument Control* tab. On the menu bar click *View → System Diagram*. You can mouseover system components in a yellow state to get a description of the status

Viewing Signal During Data Acquisition

1. Click on the *Instrument Control* tab. If you don't see the signal trace, on the menu bar click *View → Online Signals → Signal Window 1*
2. In the *Online Plot* window click *Change* and set up the window appropriately for your experiment.

Data Analysis and Exporting Data

1. Click on *Data Analysis* in the lower left corner. You can double click on the samples in your sequence to bring up the signal traces.
2. After bringing up the data you want to subtract the blank from, on the menu bar click *File → Subtract Blank Run*. Select the data file for your blank and click *OK*.
3. Click to integrate the peaks in the background subtracted chromatogram
4. Click [Integration] to bring up the detected peaks and their parameters such as height, area, and area%. You can use the tools above the signal trace to alter, add or remove peaks.
5. To generate a pdf report click

6. To export your chromatogram or peak integration data in csv format for processing in Excel or other software, on the menu bar click *File → Export File → CSV File*

When You Are Finished

1. Close the instrument control software and say yes to the prompts.
2. Close the control panel software.
3. Switch off the chromatograph and the column thermostat.
4. If solvents are running low or the waste container is full, notify RIC staff.
5. Log out of the PC.