Agilent 1200 HPLC and API 4000 mass spectrometer: Standard Operating Protocol

Is this the instrument I need?

What is it you want to do?

If you are looking for a sensitive, specific method to separate and detect components from a liquid sample (or extract), the Agilent 1200 and API 4000 may help. It provides good specificity, sensitivity, and linear range for targeted analysis (especially of small molecules).

Specificity (and some structural information) is provided both by the HPLC and by the mass spectrometer's modes of operation: multiple reaction monitoring, product ion mode, precursor ion mode, and neutral loss scan. However, because the mass spectrometer is limited to unit mass resolution, you will not be able to collect high mass resolution information.

Before proceeding any further, ask yourself a few questions.

1. Is my analyte ionizable? (We have both an electrospray and an APCI source.)
2. Is my sample liquid? (Please do not try to inject any sample with particulates into the LC. The person charged with maintaining it will not thank you.)
3. Will my analyte's mass-to-charge fall within the instrument's working range?

Where can I find the instrument?

The LC/MS system is sitting in AIMS (LM 18). Our bay is the second from the right and the second from the back (i.e., the side of the room opposite the door).

How do I use the instrument?

In general, the instrument is always left in standby mode.

To begin, change the mobile phase reservoirs to your desired mobile phase (if necessary). Open the purge valve, and flush the lines with each new mobile phase for five minutes, at a flow rate of 1 mL/min. Close the valve when you are done.

Equilibrate the column at your initial mobile phase composition and temperature. Keep an eye on the pressure and make sure it does not rise above either the column or the pump's maximum rated pressure. Check that the autosampler is maintaining its setpoint temperature.

Transfer your sample to LC vials. Place the vials within the sample tray, taking note of their positions, and place the tray within the autosampler.

Set up your LC method and your sample queue. Double check the positions of your samples. (If the autosampler tries to inject from a position with no vial, it will error out and you will have to restart the LC stack.)

Check the length of your sample queue, and ensure that you have more than enough solvent for the entire queue, given the flow rate of your method. *Do not let the solvent reservoirs run dry.* Though the instrument will eventually stop running after the last sample, there is an approximately half-hour delay. Make sure you account for this as well.

When your column is completely equilibrated, start your queue.

How do I keep myself and others safe?

First and foremost, **do not defeat the interlocks!**

After that, there are four primary ways one might get hurt using the instrument: 1. Solvent spraying or leaking from the pressurized HPLC flow (or LC components popping due to pressure buildup) 2. Electric shock or thermal burn from ion source 3. Turbo pump failure 4. UV exposure from DAD lamp

HPLC flow and pressure

When the pump is operating, mobile phase is being forced through the LC system at high pressure. If you disconnect any components in the flow path, the pump will continue pumping, the pressure will contain pressing, and solvent or eluent will spray out of the free end! Always turn off the pump before disconnecting pieces. (As always, you should be wearing safety glasses and gloves.)

Sometimes, even if you have not intentionally disconnected anything, a loose connection will cause a leak. You may notice the growing puddle yourself (always check for this if the pressure is inexplicably low) or the leak may trigger the leak sensors and disable the pump. Use normal chemical spill procedures to clean up the spill. Be sure to thoroughly wipe the area around the leak sensors, or the instrument will not restart.

Finally, you may encounter the opposite problem: rapidly building pressure due to a clog in the system. The HPLC is usually set to disable when the pressure reaches a critical threshold (either 600 bar or the pressure threshold of your column, whichever is lower). But don't rely on this! If the pressure is rapidly climbing to the threshold, turn off the pump! Then start disconnecting pieces to isolate the location of the blockage.

Ion source

The ion source presents the twin dangers of high voltage and high temperature. Fortunately, the instrument comes with safety interlocks to protect you, but you should still follow these precautions:

Before changing the ion source probe, disable the instrument through Analyst. (Click Hardware Configuration, then select the currently active configuration and click Disable.)

Do the same if you need to remove the ion source chassis, but also wait for it to cool down.

Turbo pump

The turbo pump usually operates without any need for special concern, but it may fail. If you notice a high-pitched shrieking or screeching (think nails on chalkboard), turn off the instrument. See the last section for how to do so.

DAD lamp

The Agilent 1200 includes a diode array detector, which can be used as the HPLC detector in addition to, or instead of, the mass spectrometer. This DAD, which is the module on the top of the right hand stack, uses a UV lamp as a light source. Do not open up the DAD without first turning it off and disconnecting it, to avoid exposure to ultraviolet radiation.

If in doubt, don't DIY.

Remember:

This is not an exhaustive list! Exercise caution and common sense. (I.e., do you want to stick your hand in the autosampler as the needle is moving? No. Do you want to tilt the mass spectrometer and drop it on yourself? Also no.)

What do I do in an emergency?

To stop operation, disable the hardware through Analyst.

If you must turn off the mass spectrometer, find the power switch on the right hand side of the instrument, and turn it off.

We usually leave the rough pump running for half an hour after shutdown, to provide backing vacuum as the turbo pump spins down. (This is kinder to the turbo pump, and safer for you!) But if you must turn off the rough pump

immediately, open the large compartment on the mass spectrometer table. Find the power switch on the right hand side of the rough pump, and turn it off as well.

To turn off the HPLC, press the power switches on the lower left hand corner of each module.