



## OPERATING INSTRUCTIONS FOR USE OF THE HPLC (HEWLETT PACKARD 3390A INTEGRATOR, SPECTRA-PHYSICS ISOCHROM PUMP, AND SPECTRAFLOW 783 ABSORBANCE DETECTOR)

High-Performance Liquid Chromatography is a type of column chromatography used to separate components of a mixture by noncovalent chemical interactions between the analyte of interest and the chromatography column. An analyte that acts less favorably with the column will be eluted faster than an analyte that acts more favorably with the column. The time at which the analyte elutes from the column is known as the retention time (RT); retention times can be compared to qualitatively identify a particular analyte. The Hewlett Packard integrator creates a chromatogram based on the UV detector signal as each analyte elutes from the column; the signal (peak area) is proportional to the amount of that component thus providing useful quantitative information. It is important to choose the appropriate calibration method (internal standard or external standard) for your application.

The basic capabilities of this instrument and operating procedures are covered in this document. More advanced procedures can be found in the literature, and the following references may also be of use:

- For hardware problems regarding the HPLC pump, consult the SP8800/8810 LC Pump Operators Manual
- For problems associated with the integrator, refer to the 3390 Reporting Integrator Quick Reference Guide
- For a more advanced overview of HPLC consult:
  - CH 28, "Liquid Chromatography" in *Principles of Instrumental Analysis*, 6<sup>th</sup> Ed. By Skoog, Holler, and Crouch
  - CH 25, "High-Performance Liquid Chromatography" in *Quantitative Chemical Analysis*, 6<sup>th</sup> Ed. By Harris

### Operating Procedures:

**1. Set up:** Before powering on the HPLC, be sure that the mobile phase bottle is filled with the mobile phase of choice. If the mobile phase falls below a certain level, it is possible to introduce bubbles into the system. If bubbles are introduced into the system (which also could result from lack of use), the system will need to be purged as described later. To power the HPLC pump, press the on/off switch located on the right-hand rear panel of the instrument. The parameter that may need to be adjusted at this point is the flow rate. In order to change it, press the flow button, and then using the numbers on the keypad, enter the desired flow rate followed by the enter button. At this point, the pumps should begin running. If at this time or any later time the pressure exceeds that maximum operating pressure, immediately press stop and purge the system.

Locate the toggle switch on the UV absorbance detector and depress. To change the wavelength, press the lambda ( $\lambda$ ) button, and then using the numbers on the keypad, enter the wavelength desired followed by the enter button. After adequate time has passed to assure that the column has been flushed with your mobile phase (*i.e.*, 15-20 minutes), be certain to zero the instrument by pressing the auto zero button. The detector will say "detector ready" if everything is working properly.

In addition to the HPLC and detector, there is an integrator for which the parameters need to be set. To power the integrator, press the on/off switch located behind the instrument. Several parameters can be adjusted: Attenuation (ATT), chart speed (CHT SP), peak width (PK WD), and threshold (THRSH). A typical set of parameters is as follows: ATT = 5, CHT SP = 0.5, PK WD = 0.16 and THRSH = -2. These parameters are usually adjusted based on separation and clarity of display once chromatograms have been collected. To enter desired values, press the button corresponding to the parameter you wish to adjust, enter the value using the numeric keypad, and press enter. To verify a current parameter setting, press the button corresponding to the parameter you wish to determine, and hit the LIST button.

**2. Injection:** To ready the instrument for sample injection, press the enter button on the key pad to raise the pressure. When the HPLC is stable, a red “ready” light will appear. If pressure seems to be inconsistent and the ready light never appears, attempt to purge the system as described later. Allow the HPLC to run with the ready light on for 10 -20 minutes to ensure that anything left on the column will be washed away and not show up as an unidentified peak on the next chromatogram. Locate the black lever and make sure it is in the load position (up). Using a syringe, load the sample in the injector port. There is a sample loop that holds only 10 µl, so be certain to add slightly more than you need. This will be apparent when an excess is observed leaving the 10 µl loop and being collected in the waste beaker. Only ten µl of the sample is injected into the column. After the sample has been loaded, get ready to pull the black lever to the down position (inject). The lever and the start button on the integrator need to be pulled/pushed simultaneously and consistently for the rest of the injections. Inconsistency will result in poor reproducibility of retention times.

**3. Record Keeping:** As a general rule, you will wish to include a copy of each chromatogram in your laboratory notebook as well as the following parameters:

<b>Column</b>	Brand, Type, Particle Size, Dimensions
<b>Column Temperature</b>	Very important if not at room temperature
<b>Mobile Phase</b>	Ratio of components
<b>Flow Rate</b>	Typically in mL/minute
<b>Back Pressure</b>	Typically in MPa or PSI
<b>Detector</b>	Type (e.g., UV) and important settings (e.g. wavelength)
<b>Derivatizing Agent</b>	If applicable
<b>Standardization Method</b>	e.g., external standard, internal standard
<b>Recorder Attenuation</b>	Typically in the range of -5 to 5

**4. Purging the System:** If you find it necessary to purge the system, obtain a beaker and locate the purge button and purge valve (black valve connected to plastic tubing). Turn the purge valve to the left to open it and place the beaker under it so as to catch the mobile phase as it leaves the system. Press the purge button. If bubbles are present, they will be noticeable coming out of the system. After bubbles have disappeared, press the stop button and close the purge valve. Discard any organic-based solvents in the appropriately labeled waste container.