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Title:	**Waters HPLC Start-up Procedure**		
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Introduction

High-Performance Liquid Chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. Our Waters HPLC system consists of Binary HPLC pump 1525, Dual λ absorbance detector 2487, Multi λ fluorescence detector 2475 and Electrochemical detector 2465.

Associated Procedure

Guidelines

1. Power on Pump. Ensure all solvent inlet lines are in fresh solvent.
2. Determine which detector will be needed for your separation and make proper fluidic connections to and from them. If using the 2487 TUV detector and/or 2475 FLR detector (s), do not power them on yet.
3. Prime the solvents by opening the silver Vent Valve on the pump by turning the handle to the right. Then set the flow rate on both Pump A and Pump B to 5 mL/min each via Empower.
4. You may assist the priming by rotating the black Purge Valve on each pump counterclockwise one rotation and drawing solvent through the port in the middle with a syringe. When no air bubbles are observed retighten the Purge Valve.
5. When confident no air gaps are in the solvent lines and no bubbles are present when priming set the flow rate on both Pump A and Pump B to 0 mL/min. Set the flow rate and composition on the pump to your initial conditions for the separation. Then close the silver Vent Valve on the pump by turning it all the way to
6. Ensure that all the sections of tubing (especially in the Column Heater) are connected as solvent is flowing and will leak through any unconnected sections.
7. Once solvent has flowed through the system for several minutes, attach your column in the Column Heater Module. Feel free to stop the flow to minimize any solvent leaks and restart flow once the column is connected. At this point you can manually enter your desired column heater temperature on the Temperature Control Module.
8. With mobile phase passing through the detector(s) of choice you may now power them on and allow them warm up and fully initialize.
9. Run samples through Empower control.
10. If any buffers or salts have been used for the separation: ensure that the entire system

has been flushed for several minutes with both solvent lines A and B in a mobile phase of 50% Methanol and 50% Water (disconnect the column if this mobile phase is incompatible with its chemistry). Follow the procedure in Steps 2 and 3 to prime the solvent.

11. After separation is completed and buffers (if used) are flushed, power down all modules. the left.

Revision Number	Contact