

LC6000 Good Practice Handbook



Introduction

The LC6000 Good Practice Handbook is designed for all users of SCION Instruments LC6000, High Performance Liquid Chromatography (HPLC) systems. The below set of guidelines will ensure reliability and efficiency of the LC6000.

A. Mobile Phase

Optimisation of the mobile phase is critical to not only any HPLC application but for maintaining the health of your LC6000. Careful consideration is needed during mobile phase selection.

A1. Solvent Quality

Any solvents as mobile phase should be of the highest HPLC or MS grade quality. The solvents should not contain any impurities which could lead to contamination, ghost peaks visible in results and pressure fluctuations within the LC6000. Aqueous solvents should always be deionised and purified.

A2. Solvent Selection

Solvent selection is an intrinsic part of method development and optimisation. The elution strength of selected solvents will determine the separation of both target compounds and method run time. By choosing a solvent with a strong elution strength, typically Acetonitrile, a lower solvent to aqueous ratio is required, thus reducing cost. However, consideration must be taken as interactions between functional groups of a target compound and both the stationary phase / mobile phase can occur. Additionally, when using an Ultra-Violet detector, Diode Array Detector or Fluorescence Detector, the specific absorbance of the solvent must also be considered. If the absorbance of the solvent is similar to that of the target compound, then the solvent is not suitable for use as mobile phase.

A3. Use of Buffers

Mobile phases containing a buffered solution should be prepared fresh daily. Buffers should be homogeneous, fully dissolved and pH adjusted accordingly. Poor pH control can lead to damage of the LC6000 and analytical column. When using a buffered mobile phase, always use a plunger wash on the 6100 Pump as this will eliminate the build-up of any salts within the buffer. At the end of the day always flush the system with water and solvent. This will prevent salt precipitation, damage to the instrument seals and leaks within the system.

A4. Filtering of Mobile Phase

Offline filtering of the mobile phase is recommended especially when buffered solutions have been prepared, by using a 0.45µm filter. This will remove any particulates from the mobile phase, preventing blockages of the LC6000. Solvent bottle filtering (online) is always recommended, especially when a highly aqueous mobile phase is used. The LC6000 is supplied with online mobile phase filters, connected to the mobile phase PTFE tubing. It is good practice to regularly change these filters from the solvent bottle.

A5. Degassing of Mobile Phase

Degassing of the mobile phase is critical to any HPLC setup. Degassing removes air from the mobile phase, preventing blockages within the LC6000. If air is introduced into the 6100 pump, unstable flow and pressure fluctuations will be observed. Remove air from the LC6000 by purging the system. It is always recommended to use the 6-Channel Degassing Unit with the 6100 pump, however offline manual degassing is also recommended.

A6. Other Mobile Phase Considerations

When choosing your optimum mobile phase for the analysis, be sure to consider the pH limitations of your analytical column. Incorrect pH of the mobile phase can damage the stationary phase of the column, resulting in poor chromatography and shorter lifespan of the column. When using an aqueous mobile phase, regularly flush the column with solvent to prevent microbial growth. It is good practice that when storing a column, it is thoroughly flushed with organic solvent and correctly capped at each end.

B. Sample Preparation

The removal of interferences and sample matrices is vital prior to any analysis being completed on the LC6000. This process is done through sample preparation. Alongside the removal of any interferences, sample preparation is also completed to dilute or concentrate samples. Sample preparation techniques such as Liquid-Liquid Extract, Solid Phase Extraction and sample filtering, ensure clean samples are introduced into the HPLC which also protects the stationary phase of the column.

B1. Solubility of the Sample

To eliminate split or broadened peaks, the sample must be completely soluble in the chosen solvent. The chemical and physical properties of target compounds should be used to determine their solubility. If the solubility of the sample is unknown, a solubility test must be performed. Dissolve the sample in different solvents and observe the solubility behaviour during each test. If the sample is soluble in a solvent, the end solution will be clear and free from particles.

B2. Solvent Selection for Sample Preparation

When preparing a sample for analysis, it is important to ensure that the sample is dissolved or prepared in the same solvent as the mobile phase. In particular, if a gradient mobile phase method is used, the sample should be prepared in the same composition as the start of a method. For example: If a gradient method begins at 15% methanol: 85% water, the sample should also be dissolved in 15% methanol: 85% methanol.

C. Instrument Preparation

Before performing an injection on the LC6000, ensure that the instrument is ready for use.

C1. Instrument Warmup

Before beginning an analysis, ensure that the lamps of the detectors are turned on and have had sufficient time to heat up. The lamps are ready to use when the baseline of the LC6000 is stable. Flush the LC6000 with solvent to ensure that there is no air in the system. If fluctuating pressure or flow is observed then use the Purge function within the Maintenance Tool. Once the system is free of air, set the pump to a flow of 1mL/min and observe for any leaks. Continue flow through the column to ensure the column is equilibrated with the current mobile phase (flush the column with 10 to 20 times the column volume for proper equilibration).

C2. Instrument Checklist

Before beginning an analysis ensure that all of the below requirements are met

- All LC6000 modules are connected in series to the organiser
- All tubing and connections are secure with no leaks
- The instrument is configured within Compass Configuration Manager and communication has been established
- Detectors are turned on, lamps are heated before use

- Fresh mobile phase has been prepared including degassing
- The LC6000 has been properly purged with new mobile phase
- Purging of the LC6000 has been performed correctly and no air is in the system
- The autosampler syringe is free from air
- HPLC column has been installed and flushed thoroughly with fresh mobile phase

If the above steps have been completed, the instrument is ready for use.

D. Instrument Shut Down

When an instrument is to be shut down, it is critical that it is done in a manner that the instrument will not be damaged.

D1. Shut Down Procedure

- Ensure that all analyses have been completed
- Flush the column with solvent to ensure removal of contaminants
- Disconnect the column and install a backpressure device
- Remove all samples from the autosampler tray
- Flush the system with water if a buffered solution was previously used
- Replace the mobile phase with 100% Methanol or Isopropanol
- Purge the pump with the above solvent before flushing the system at 1mL/min (ensure no column is installed)
- Wash the needle two to three times
- Disconnect the system from CompassCDS control and close CompassCDS

If the above steps have been completed, the instrument is ready for switch off. Always turn off the power to each individual module before turning off the power to the Organiser.

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