# LC-MS Checklist

## Before the Analysis

### Sample Preparation

* Filter samples before injection with a Syringe and Filter.
  + Syringe filters can be found in the cupboard in the weighing room.
  + Clean syringes can be found in sealed packets in the cupboard under the HPLC.

### Pre-Run

1. Check the indicator lights on the Autosampler, Pump, Column, VWD and MS are not Red.

Orange, Flashing Orange or Off are OK.

1. Check the valve near the ESI on the MS is open (handle in line with pipe) and is connected to L on the nearby block (shown below)

Check that the pipe is connected to the port marked “L” to the left of this image, not to the port marked “H”

1. Check the levels of the bottles on the front of the MS. Tap the bottles to disturb the liquid if the level is not obvious.
2. Check the levels of the waste bottles in the following locations:
   * Beneath the bench below the Pump/Autosampler/Eluent stack
   * Beneath the bench below the MS and behind the Vacuum Pumps
   * Behind the MS
3. Check there is pressure in the Nitrogen Bottle behind the MS
4. Check the 3 Exhaust Pipes are entering the Extractor for the Graphite Furnace
5. Return to the front of the instrument
6. Fetch some blue towel.
7. Open the Ionisation Chamber of the MS and spray 50% IPA around (but not into) the metal surfaces of the inlet orifice.
8. Spray the inside of the Ionisation Chamber door and wipe with the blue towel.
9. Close the Ionisation Chamber.
10. Open Mass Hunter Workstation Data Acquisition.
11. Check the pressures on the top right of the screen are within the following values:
12. Check sufficient eluent is present in the eluent bottles being used for the run.
13. Check that the Quat. Pump is aware of the level of eluent in the bottles. To do this:
    * Right click on the Quat. Pump panel of the Instrument Status window in MassHunter. <could use image here>
    * Select Bottle Fillings from the menu.
    * Under Actual Volume, ensure the value reflects the volume of eluent actually present in the bottle. <could use image here>
    * Click OK.

Repeat the above steps when Changing Eluent (see below)

### Changing Eluent

When changing eluents, try to swap similar eluents. For example, replace Ammonium Formate with Ammonium Acetate.

* The Lids on the bottles do not need to be tight.

To change an eluent, perform the following steps:

1. Right click on the QTOF panel of the Instrument Status window in MassHunter and select LC → Waste from the menu.

The Instrument Status window

1. In the Method Editor, under Quat. Pump, set the Flow Rate to 0.000 mL/min and press Enter⏎
2. Open the valve behind the door on the front of the Pump.
3. Set the level of any solvents that weren’t changed to 0% and enable the eluents that were changed.
4. Set the Flow Rate to 1.000 mL/min and press Enter⏎
5. Check the pressure in the Column does not rise above 0 Bar. If it does, check the valve at the front of the Pump is open.
6. Set the Flow Rate to 5.000 mL/min and press Enter⏎
7. After 5 minutes, set the Flow Rate to 0.000 mL/min and press Enter⏎
8. Close the valve on the front of the Pump and close the door.

A method can be setup for flushing and equilibrating the column as part of the Worklist if desired

## Mass Calibration / Check

1. Select Context → Tune at the top left of the MassHunter window.
2. Turn on the QTOF by clicking the On button in its section of the MassHunter window.
3. Close the valve near the ESI on the MS.
4. Set Calibrant Bottle to B at the bottom left of the Tune window.
5. Set LC Flow to to Waste at the bottom left of the Tune window.
6. In the Tune & Calibration tab (pictured below) set the instrument parameters required for the run, e.g.:
   * Positive – if using +ESI
   * TOF - <when would you use both?>
   * Mass Calibration / Check
   * 50-1500 m/z

The Tune & Calibration tab

From Agilent’s manual:

Before you calibrate the instrument, you have to set the instrument state

to the proper instrument mode, mass range and fast polarity switching

mode. You set these values on the Instrument State tab.

When you change the mass range or enable/disable fast polarity switching

on the Instrument State tab, the pulser frequency is changed which results

in the DEI pulser warming up or cooling down. If the calibration is

performed too soon, the DEI may still be heating up or cooling down which

can result in drift. See the online Help for more information on the

Instrument State tab.

1. Click the button to start the calibration.
2. Once complete, the calibration report will open automatically.
3. Under TOF Mass Calibration Data, for the largest mass check that the resolution is at least 20,000 and that the corrected residuals is below 5 ppm.
4. On Page 2 of the report, under Detector → MCP, check that the value is below 900.
5. Back in MassHunter, return to Acquistision mode by selecting Context → Acquisition at the top left of the window.
6. Open the valve near the ESI on the MS.

## Performing Analysis

### Starting a Run

If Mass Calibration / Check was performed the LC flow will have been set to go to the MS automatically. Otherwise, perform the following steps to set the flow:

1. Right click on the QTOF panel of the Instrument Status window in MassHunter
2. Select LC → Waste from the menu.

The Instrument Status window

To start the run, in the Worklist, select the samples to run by ticking the box to the left of the Sample Name field and click the ▶ button.

The Worklist

### During the Run

1. Check that the Column pressure remains stable and does not rise above <value?>.
2. Check that the three symbols in the Q-TOF panel of the Instrument Status window (shown below) remain green to indicate that the reference ions were found.

The Instrument Status window