LC-MS User Guide

Release 1.0.0

Dominic Davis-Foster

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CHAPTER 1

Before the Analysis

1.1 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`.

1.2 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.
2. 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)
3. 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.
4. 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`
5. Check there is pressure in the `Collision Gas Bottle` behind the `MS`, and record the pressure
6. Check the 3 `Exhaust Pipes` are entering the `Extractor` for the `Graphite Furnace`
7. Return to the front of the instrument
8. Fetch some blue towel.

Fig. 1: Check that the pipe is connected to the port marked "L" to the left of this image, not to the port marked "H"

9. Open the `Ionisation Chamber` of the `MS` and spray `50% IPA` around (but not into) the metal surfaces of the inlet orifice.
10. Spray the inside of the `Ionisation Chamber` door and wipe with the blue towel.
11. Close the `Ionisation Chamber`.
12. Open *Mass Hunter Workstation Data Acquisition*.
13. Check the pressures on the top right of the screen are within the following values:

Rough Vac	< 4.4×10^{0}
Quad Vac	1×10^{5} - 4×10^{5}
TOC Vac	2×10^{7} - 4×10^{7}

14. Check sufficient eluent is present in the eluent bottles being used for the run.
15. Check that the `Quat. Pump` is aware of the level of eluent in the bottles. To do this:
    1. Right click on the `Quat. Pump` panel of the *Instrument Status* window in *MassHunter*. <could use image here>
    2. Select *Bottle Fillings* from the menu.
    3. Under *Actual Volume*, ensure the value reflects the volume of eluent actually present in the bottle.
    4. Click *OK*.

    Repeat the above steps when Changing Eluent (see below)

1.3 Changing Eluent

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.
2. In the *Method Editor*, under `Quat. Pump`, set the *Flow Rate* to 0.000 mL/min and press `Enter`

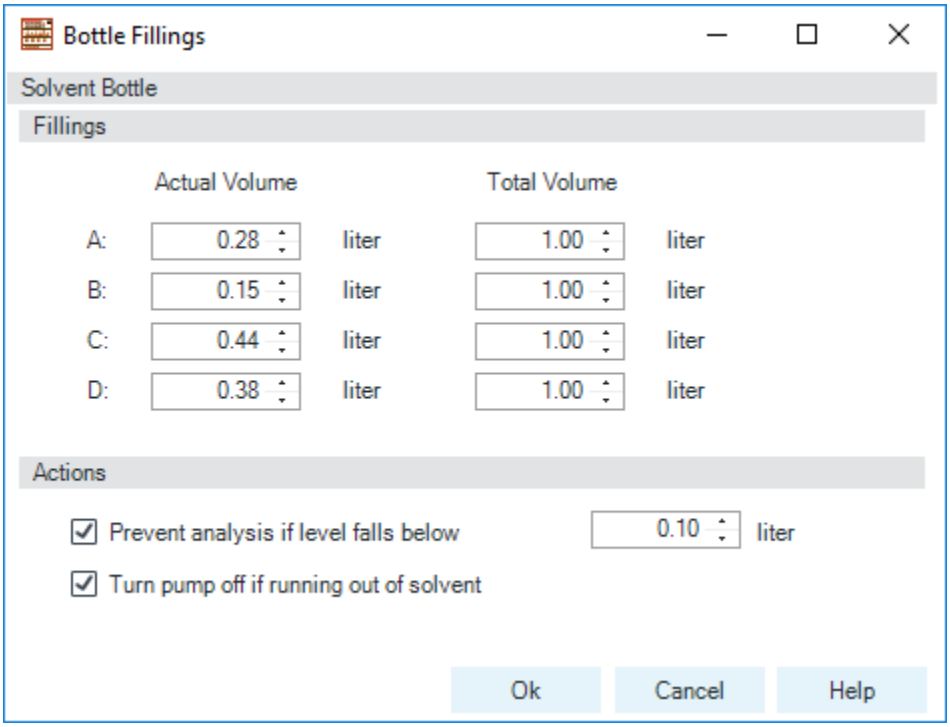

Fig. 2: The “Bottle Fillings” window

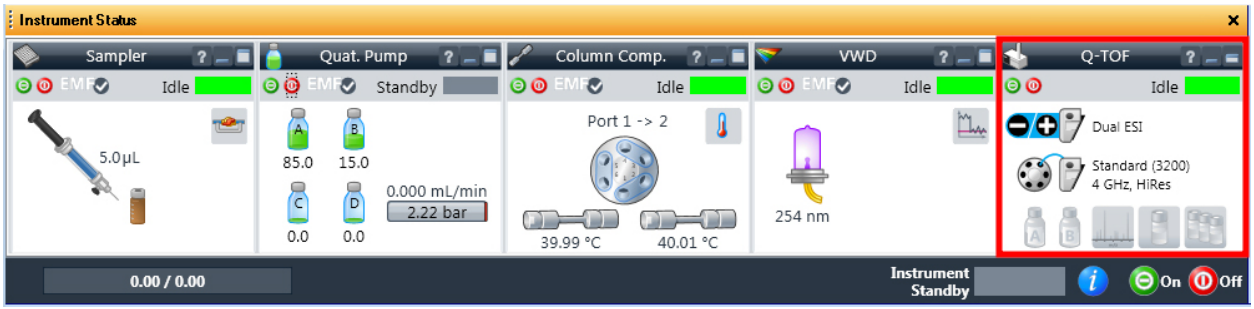

Fig. 3: The Instrument Status window

3. Swap the the eluent bottles. Try to swap similar eluents; for example, replace *Ammonium Formate* with *Ammonium Acetate*.
    - The Lids on the bottles do not need to be tight.
4. Open the valve behind the door on the front of the `Pump`.
5. Set the level of any solvents that weren't changed to 0% and enable the eluents that were changed.
6. Set the *Flow Rate* to 1.000 mL/min and press `Enter`
7. 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.
8. Set the *Flow Rate* to 5.000 mL/min and press `Enter`
9. After 5 minutes, set the *Flow Rate* to 0.000 mL/min and press `Enter`
10. 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

CHAPTER 2

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*

   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.
   ```

7. Click the button to start the calibration.

   Note: The text of this button veries depending on the mode selected.

   - For *Quadrupole*, the button reads *Start Check Quadrupole*

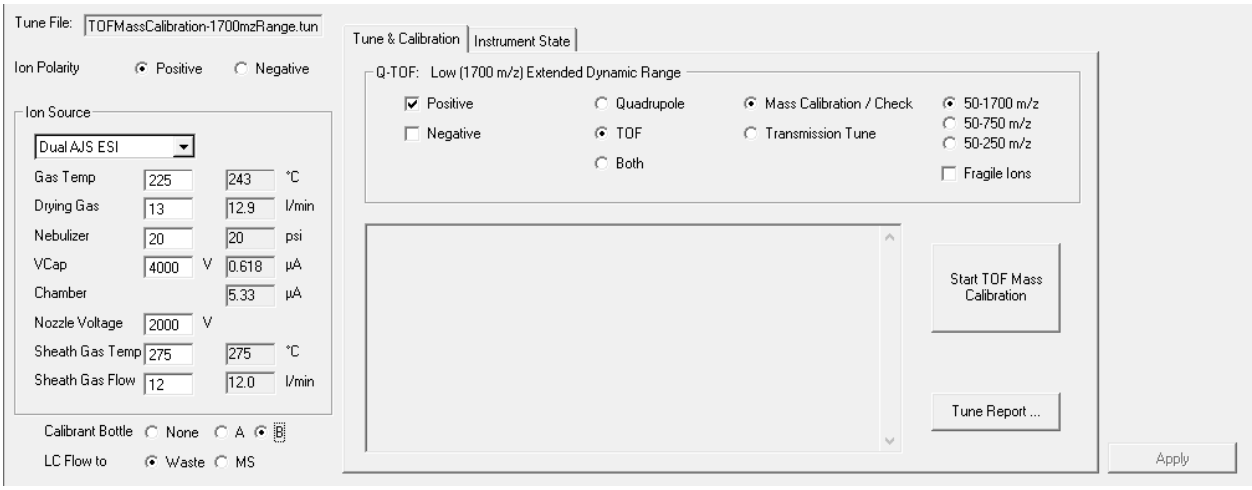

Fig. 1: The Tune & Calibration tab

- For *TOF*, the button reads *Start TOF Mass Calibration*
- For *Both*, the button reads *Start Check Quadrupole & TOF Mass Calibration*

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8. Once complete, the calibration report will open automatically.
9. 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.
10. On Page 2 of the report, under *Detector* → *MCP*, check that the value is below 900.
11. Back in *MassHunter*, return to *Acquisition* mode by selecting *Context* → *Acquisition* at the top left of the window.
12. Open the valve near the `ESI` on the `MS`.

CHAPTER 3

Performing Analysis

3.1 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.

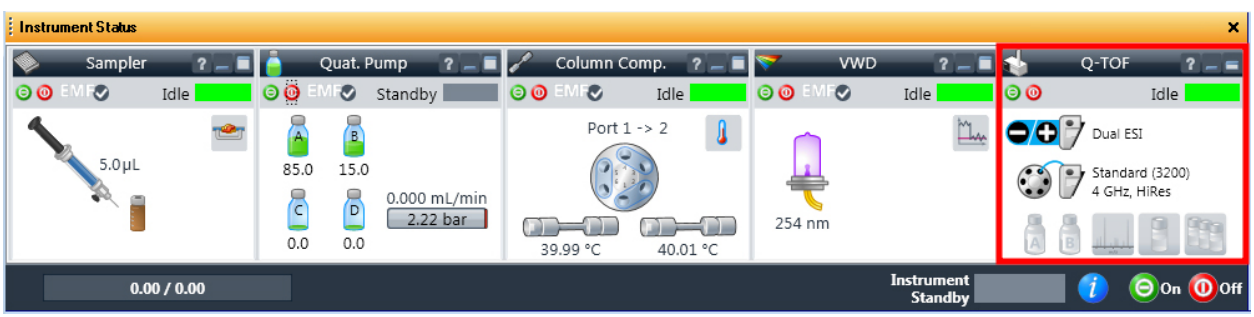

Fig. 1: The Instrument Status window

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.

3.2 During the Run

1. Check that the `Column` pressure remains stable and does not rise above 200 bar. Analysis should stop automatically if the pressure rises above 600 bar.

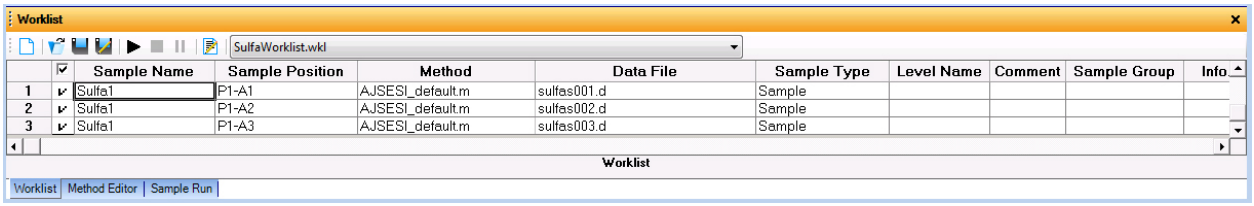

Fig. 2: The Worklist

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.

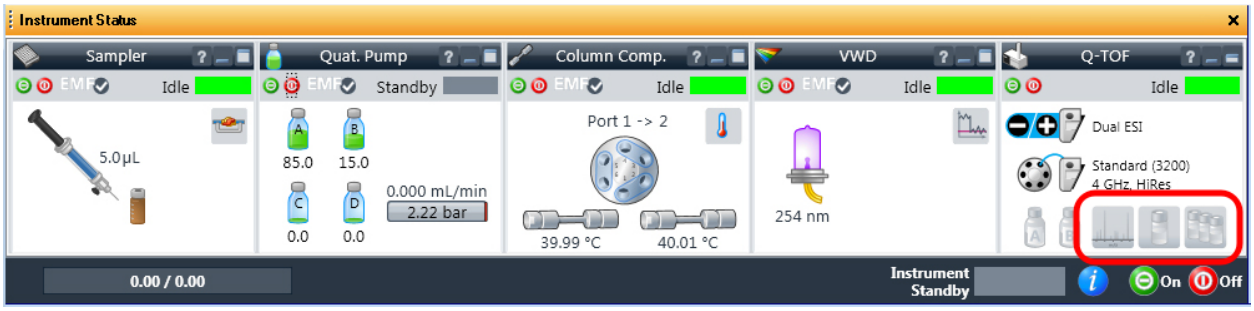

Fig. 3: The Instrument Status window

3.3 Shutdown

1. In the `Instrument Status` window, click the *Off* button to the lower right.
2. Close `Data Acquisition`.