Training Prerequisites

Mass spectrometry equipment is expensive, poses electrical hazards. While the automated operation excludes many of the steps that may cause damage, the users must still follow a certain protocol when preparing and submitting their samples and avoid improvising in unfamiliar situations. Please read the following before attending MS training session. You will be first usually trained to use the Waters UPLC-SQD2 and Waters UPLC-SQD. Once experienced, you will be trained to use the Agilent 6530 QTOF LC-MS/MS by the mass spectrometry staff.

Sample preparation

- It is very important you prepare your sample properly! Usage of Zeba spin desalting column is essential.
- User guide for Zeba spin desalting column protocol:

1. Zeba Spin Desalting Columns https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0011512_Zeba_Micro_Spin_DesaltCol_7K_MWCO_UG.pdf
2. Zeba Micro Spin Desalting Columns https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0011512_Zeba_Micro_Spin_DesaltCol_7K_MWCO_UG.pdf

- Solvent allowed for Mass spec submissions are water and volatile buffer (e.g., NH_4OAc, acetate buffer)
- Briefly, desalt 100 µL of 5 µM sample with Zeba spin (wash Zeba 1× with LC-MS grade water, this can remove any PEG impurity which may be in Zeba) so your **sample is in water**. Then dilute your sample to **4-5 µM**. You can also use a volatile buffer (e.g., NH_4OAc). If your sample is precious, you can use the more expensive small Zeba microspins to desalt 12 of 5 µM sample and dilute that to 0.5 µM. It is very important to **not run any non-volatile buffers** on the instrument (PBS, BBS, etc.)!
- Spin the Eppendorf with your sample for 12000 rcf for 2-5 min so any dust or particulates settle to the bottom. With a clean pipette tip, pipette 30 µL of sample from the top (!) into an MS vial. 2 µL of sample will be injected for analysis.

Your sample

To avoid instrument downtime, use only LC-MS vials/caps from the mass spectrometry laboratory. You could obtain them from LG11, please take a whole pack of 100 vials and caps and sign the number of packs taken in the consumable book.

Avoid attaching paper labels or anything else to the LC-MS vials. For small volume samples, please use Thermo LC vials and caps also available from the mass spectrometry lab. Alternately you could use inserts, which could be found in the mass spectrometry laboratory.

If the instrument is not working correctly or if you have any questions/concerns or the LC vial broken inside of the instrument, do not leave the mass spectrometry laboratory without notifying the mass spectrometry manager. Should this happen in the absence of the staff member, **e-mail the mass spectrometry manager immediately** (kersti.karu@ucl.ac.uk).

Booking the instrument time

Please book the instrument time slot (WEEKDAY/WEEKENDS included) using this booking system https://www.chem.ucl.ac.uk/resources/week.php?year=2023&month=05&day=23&area=4&room=3

The Agilent 6530 QTOF LC-MS/MS instrument requires pure nitrogen for electrospray ionisation (ESI), which supplied to an ESI source from two dewars or four nitrogen cylinders situated in LG11.

Changing N_2 Dewar

The liquid nitrogen dewars can be only changed over by the mass spectrometry laboratory manager.

In the absence of the laboratory manager, both Crosby Medley and Martyn Tower are fully trained to change liquid nitrogen dewars.

If the liquid nitrogen run out over the weekends, no-one other than the mass spectrometry manager, Crosby Medley And Martyn Towner are allowed to change over the liquid nitrogen dewar on the following Mondays.

If there is no liquid nitrogen, please notify immediately the mass spectrometry manager by e-mail or in person.

The mass spectrometry laboratory normal hours are from 9am to 5pm Monday to Friday.

If the instrument is not operating how it should be during weekend, notify the mass spectrometry manager by email immediately. Please do not attend to troubleshoot the instrument if you are working alone in LG11.

- ✓ Please do not forget to collect your analysed samples from the auto sampler of Agilent 6530 QTOF LC-MS/MS.

Checklist before clicking "On" to start instrument

1. Solvent levels "A2" and "B2" (if low, please refill – make up solution as follow: 0.1 % formic acid (2.5 mL, in fridge) into unopened LC-MS grade H_2O and MeCN (2.5 L)
2. Check blank: H_2O or 1:1 H_2O/MeCN, position P2-A11
3. Fab standard 0.5 µM (if low, contact Chudasama/Baker group) – P2-A10
4. Reference mass 1000x dilution level (if low, please refill - contact group members/Kersti)
5. N_2 levels (if empty, change Dewar or contact Kersti)

Replacing solvent levels

1. make up solution as follow: 0.1 % formic acid (2.5 mL, in fridge) into unopened LC-MS grade H2O and/or MeCN (2.5 L)
2. right click on binary pump module, set solvent levels, set to 2.5 L. This concern A2 and B2.

Calibrating and setting up instrument (Figure 1-Figure 6)

1. Click "On" to start instrument
2. Click tune
3. Under instrument state tab,
   a. for mAb, full Ab analysis, click Load – select TOFMassCalibration**20000mz**Range.tun - OK
   b. for Fab/smaller protein fragments, click Load – select TOFMassCalibration**3200mz**Range.tun – OK
   c. if the right Mass range is already selected, you can skip next step
4. Click Apply (Equilibration will start and takes 20 mins)
5. Under tune & Calibration tab, click calibrate
6. A pdf document will be generated. Save as document and under a new folder (ddmmyy format e.g., 091122).*This folder will be where data will be saved.
7. Note parameters down in Agilent 6530 QTOF logbook (the usual table parameters and mass deviation
8. Click Acquisition
9. Save as worklist (ddmmyy)
10. Click worklist parameters – change file path for saved data (second tab). *this file path should match step 6 above.
11. **Right click the script at the end of worklist – set as pending (Figure 6).****This script will put the instrument on standby at the end

Submission as the first person to submit on the day

1. Make sure the following is selected
    a. 1st sample: BLK1; position P2-A11; method: flow_to_waste.m
    b. 2nd sample: BLK2; P2-A11; SY_LC_method_20000mz.m
    c. 3rd sample: Fab; P2-A10; SY_LC_method_20000mz.m
    d. 4th sample: BLK3; P2-A11; SY_LC_method_20000mz.m
2. Add your samples with the same format.
3. "Tick" the samples that require running and **"tick" the script at end of worklist.**
4. Click "Start worklist run" to start.
5. Ideally, please wait till the first sample get injected to ensure instrument is running.

Submission when worklist is already running

1. Click pause - The instrument will pause after the current sample finish running.
2. Add your samples with the same format as above.
3. Click "Start worklist run" to start.
4. Again, please check if instrument is injecting and running.

Submission after worklist has completed running

1. Scroll to end of worklist, **right click the script at the end of worklist – set as pending (Figure 6).**
2. Add your samples with the same format as above.
3. Click "Start worklist run" to start.
4. Again, please check if instrument is injecting and running.

Images

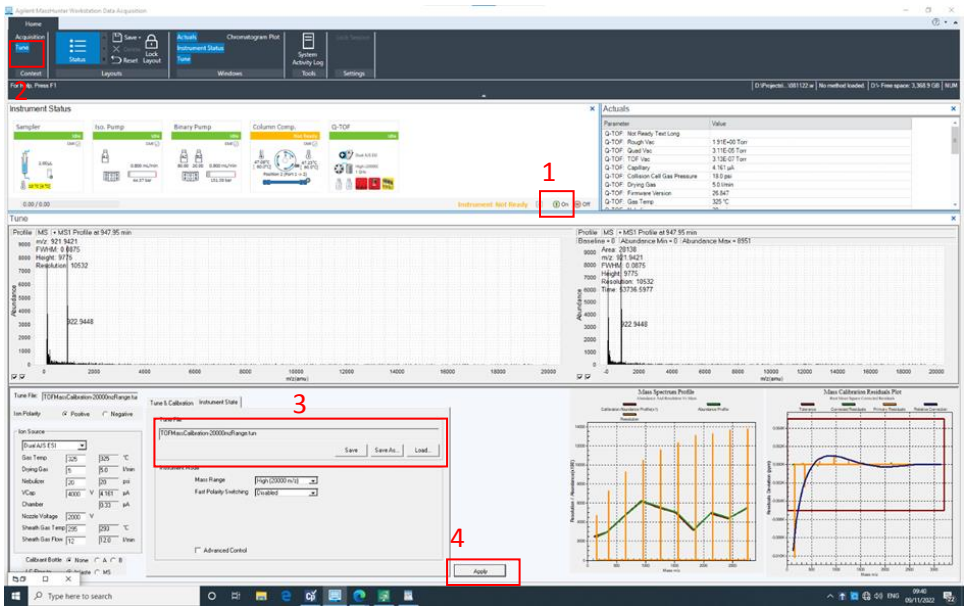

Figure 1
Calibration page for High (20000 m/z) mass range.

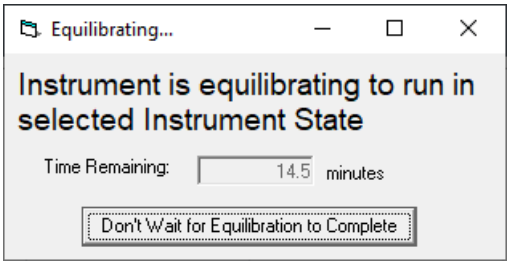

Figure 2 Instrument equilibrating after changing mass range.

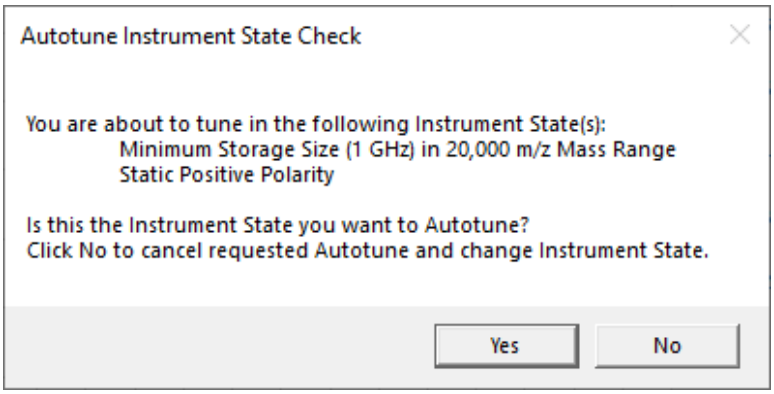

Figure 3 Autotuning instrument.

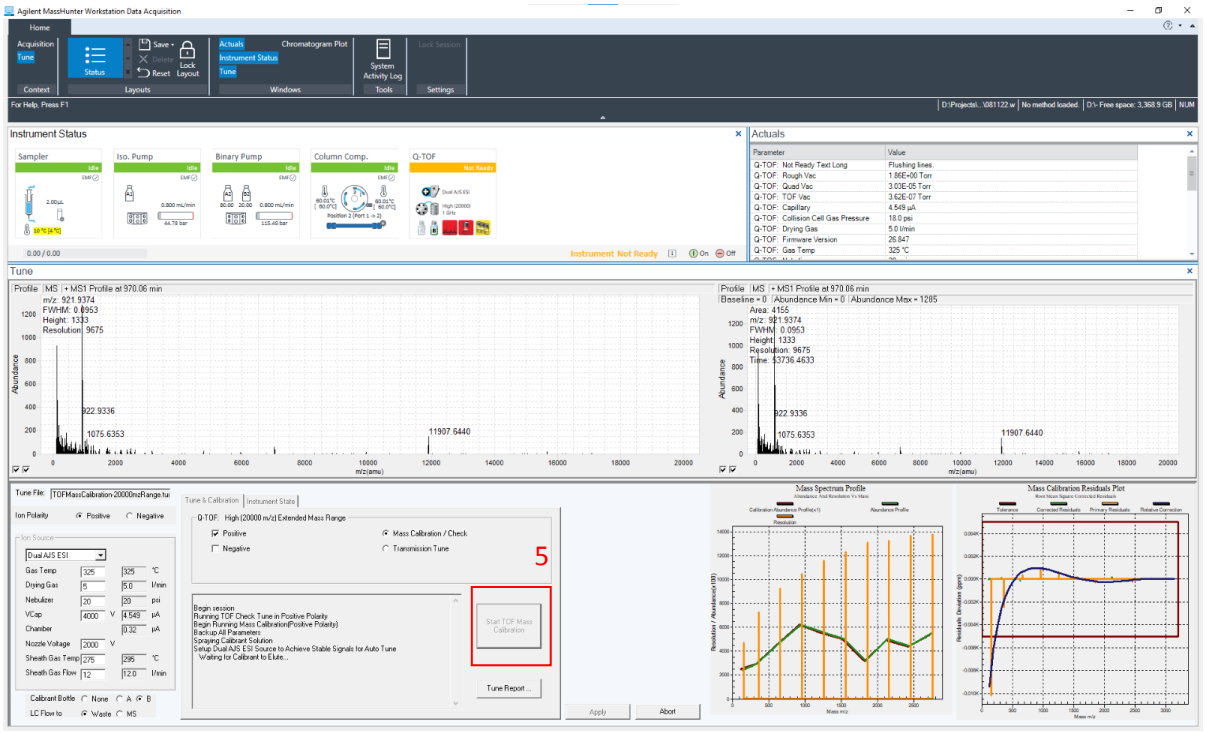

Figure 4

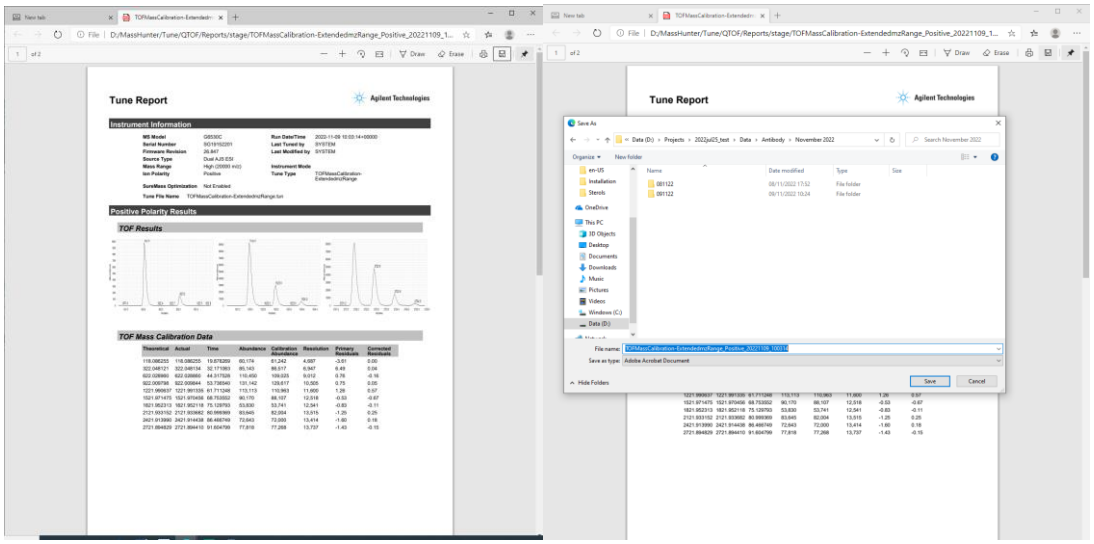

Figure 5 Save calibration report.

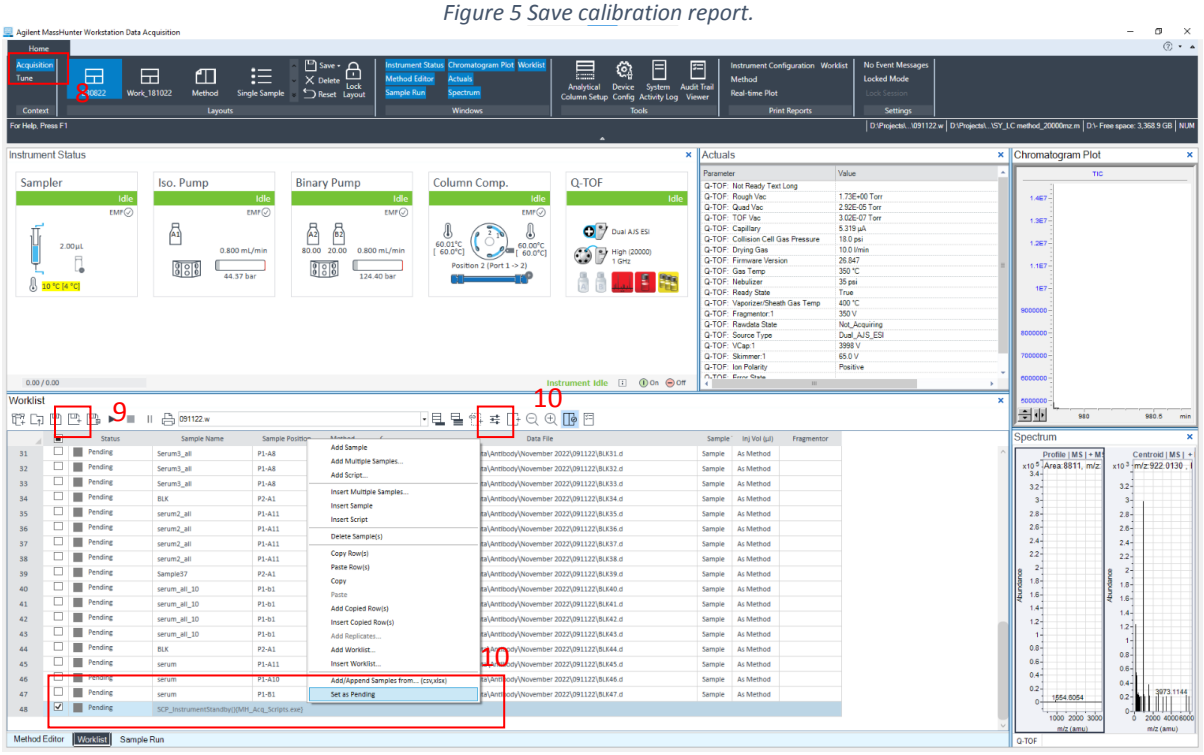

Figure 6 Make sure script at end of worklist is ticked.