Standard Operation Protocol for Waters Acquity UPLC- SQD

(Blue 'Troubleshooting' folder next to LCMS - **do not remove**)

Note: All users must obtain training from a member of UCL Mass Spectrometry Facility which includes also the Health and Safety induction for working in LG11 lab.

Also users must complete the Health and Safety Induction course which runs by Safety Officer Robert Wilson.

If you need training on using the UPLC-SQD instrument please email to kersti.karu@ucl.ac.uk

Booking time on the LC-MS system:

1. Go to: UCL Chemistry Mass Spectrometry web-site https://www.chem.ucl.ac.uk/resources/week.php?year=2022&month=07&day=15&area=4&room=3

2. Login: **Username**: First initial (CAP).surname (lower case) (i.e. S.smith), **Password**: First name (Capital 1st letter - i.e. Steve)
   - these will be setup for you when you are trained.
3. Click on 'Mass Spec', find the correct day you want and click on the time you want to book.
4. In booking info, write: Name - Supervisor - Number of samples and washes - Grant Code (i.e. Steve - JCA - 5S 2W - CEXXX)
5. In additional details: a very brief description of the sample type (i.e. short peptide w/fluorophore abs 320nm etc.)
6. Make sure the start/end times are correct. If you only need ½ hour, only book for that. **One run takes just over 5 or 10 mins**.
7. In research group: Scroll down and find the correct supervisor.
8. 'Save' - there should be two green ticks next to this. If not, you have not filled in the form correctly or are overlapping with someone else time.
9. If you finish early or do not need the time, please delete your booking ASAP so others can use the instrument and/or drop the next user a quick email in case they can run their samples sooner.
10. Please check the content of liquid nitrogen before starting any experiments. If the content is less than 5% do not start the instrumentation email mass spectrometry staff stating the content is low.

Sample preparation:

Please be very aware what you are putting through the machine. If the sample is too concentrated and/or sticky it can get stuck on the column and require lengthy washes to remove. **You are responsible for what you put through the LCMS and will be expected to clean the column if necessary**.

The column is currently a C_4. If you are worried about any samples ask around, the Macmillan and Baker groups have experience of putting larger proteins down the LCMS. If in doubt, start with a low concentration and go up.

Samples should ideally be no more concentrated than 0.1 mg/mL. If you have not observed your sample go up to 0.2 mg/mL and so on. You should not be getting TIC (total ion counts) larger than e^9.

Please make up samples in H_2O, MeOH or MeCN.

Make sure you use the correct vials and lids (we have them in the mass spec lab)

The LCMS runs gradients of MeCN/H_20 with 0.1% formic acid. **Please don't submit samples in basic buffers etc.**

If using inserts, please use the ones with the springy base. This will make sure the needle is not damaged.

Submit samples with the correct lids on.

Using the LC-MS system:

Password: there is no password

What should be open already?

1. MassLynx page
2. ACQUITY UPLC console
3. Inlet method page
4. Tune page: 'Waters SQ detector'

DO NOT close any of these pages at any point, just minimize/maximize!

If you do close any of these pages by accident, refer to the 'Troubleshooting' folder to restart.

Before start-up: Check solvent levels!

- if low (ie. <150 mL), top up. **Make sure no samples are queued to run**!
- pull out plastic stopper and both lines.
- solvents are in the cupboard next to the sink in the 'wet' lab and formic acid is in the fridge in the back room.
- solvents should contain 0.1% formic acid (ie. 500uL in 500mL). Just judge how much solvent you are adding by eye and add formic acid accordingly.
- replace stopper and both lines.
- bring up MassLynx, click on Shortcut (green arrow at top), and click on Solvent Monitor on LHS (you may have to scroll up)
- on the Overview page that opens, click the 'fill this solvent bottle' icon (the one you have filled) on the RHS
- close the Solvent Monitoring page.

If solvents have run dry, refer to the protocol in the 'Troubleshooting' folder to refill the lines.

Start-up (required if System status (bottom LHS on MassLynx page) is 'not ready'):

- open the MassLynx page, make sure the green 'Shortcut' icon is selected.
- on the LHS menu, click on 'Startup'
- Run 'Startup' sequence - click 'Yes'
- You will almost certainly see a 'Nitrogen gas failure' in the system status box. To rectify this, open the tune page ('Waters SQ detector'), click on the blue 'API cylinder icon' at the top (if it is not already depressed), then gas on the menu bar, and 'reset nitrogen failure'.
- you may have to do this a couple of times, if the nitrogen failure persists, contact John Hill.
- the Startup sequence should then run.
- once the system status is 'Ready' (green) you have 4mins to submit your sample, after which the LCMS will automatically run a shut down sequence and you have to startup again.
- ***to get consistent traces*** - make sure you wait until the pressure delta (ACQUITY UPLC console page - Binary Solvent Manager (should be selected already)) falls to around 100. This means the

pressure through the system is stable. It takes less than 1min.

Submitting samples:

- open your own project (once you are trained, a project will be created for you)

- MassLynx page, 'File', 'Open Project' and 'Save changes' to current project

- Fill in all the details for your sample

- **Filename** - whatever you like but **keep to <20 characters if you are running MaxEnt** (analysis tool for large peptides/proteins etc.) **otherwise system will crash!!!**

- File Text - leave blank if you want.

- **MS file** - this is your method editor for the MS. If you right-click on the box and select browse there should be a number of options

- eg. **std_150_2000div** (this means MS scan between 150 and 2000 masses with the first min diverted to waste)
- eg. **std_150_2000** (same as above but no divert - whole run into MS detector)
- eg. **250_2000_nodiv_con** (no divert, scan between 250 and 2000)

- you should have all of these, but if not you can set them up yourself. I would go into someone's project who has the method or similar method to what you want and copy/edit the method into your own project.

- **<u>select the MS file you want.</u>**

- if you want to see how the method is set up, select the file you want, right click and select 'edit'

- if you want to play around with settings - eg set up single ion monitoring - please save the method as something unique and understandable. **DO NOT OVERWRITE** existing methods otherwise everyone will run your method!!

- if you are changing the run time (default 5min), you will need to change the time accordingly on the MS method, otherwise the MS will turn off halfway through the run. Again save as a different (but coherent) filename - eg. **std_150_2000div_10min**

- **Inlet file -** this is your method editor for the inlet/autosampler/UV (ie. the LC part) - again right-click on the box and select browse there are a number of options.

- eg. **std_grad_5min** (5-95% MeCN over 4 min, back to 5% MeCN at 4.50min, partial loop fill, UV detection at 254nm).

- eg. **std_grad_5min_214** (same as above but with UV detection at 214)

- in this case you may not have all the methods you might want and will have to edit existing ones. Again save as something different and coherent.

- if using inserts, change the height the needle goes into the vial. Right-click on inlet file you want, click edit, click 'autosampler' (may take a few seconds to load), click 'advanced' (bottom RHS) and change Needle Placement (from bottom) to 4mm. Then save as the original method name_4mm eg. **std_grad_5min_4mm.**

- **<u>select the Inlet file you want.</u>**

- if you want to see how the method is set up, select the file you want, right click and select 'edit'

- again if you want to play around with settings please save the method as something unique and understandable. **DO NOT OVERWRITE** existing methods.

- if you change the run time, you need to change it on the Inlet/Autosampler/UV pages in the Inlet page and remember to change it on the MS method file as well (see above).Again save as a different (but coherent) filename.

- **Bottle** - where your vial is. Rack Number: Position ie. 2:F,1
- **Inject Volume** - up to 20 µL
- **MS Tune File** - 'default' should be selected

- Once all the details are filled in, highlight the row and press the blue 'Play' button.
- It will ask you if you want to 'Save changes', click 'Yes'
- Make sure 'Acquire Sample Data' box is checked and press OK.
- Sample will either be highlighted in green to show it is running, or will be placed in the queue. To see the queue, click on the 'queue' icon at the top of the MassLynx page,

Viewing chromatograms once your sample is running/has run:

- to view chromatograms, click on the 'chromatogram' button at the top of your project page in the MassLynx page.

- if your sample is currently running, you can press the 'stopwatch' icon on the toolbar to see a real-time readout of the UV and MS.

- if you want to load up an old chromatogram, 'File', 'Open' , find the file in your Project Data folder.

- when you highlight the file, you have the option of choosing the Function (ie. UV/ES+/ES-). Choose the one you want, if you want to see two spectra of the same sample, just 'File', "Open' again and select the desired function. The two specta will stack on top of each other.

- to see what masses correspond to a specific peak on the MS, right-click and drag over the relevant peak.

- there are various options to clean up and analyse the MS spectra across the toolbar at the top - have a play around if you like.

- click Print - make sure the PCL6 printer is selected. Printer is under the MALDI behind you.

Final wash cycle:

- before you leave, setup a final 'wash' (even if you are running additional washes), in the WASHLOG project.

- in MassLynx page, 'File', 'Open Project', 'WASHLOG'

- make sure the vial in position 2:F,8 has enough MeOH in it, and submit a sample at the bottom of the list with your name, date and time.

- this ensures we have some record of the state of the column and can quickly find out what, if anything, is stuck on there.

Finishing:

- You don't have to sit and wait for your samples to run. The LC-MS will automatically shut-down after the LC-MS run so you can leave it running.
- check that data come through to ms(\\storage.chem.ucl.ac.uk) if your data are not there you

need to check if the instrumentation is switched off, please go to LG11 and check if the nitrogen and mobile phase flow are switch off.

Problems:

- If you are unsure about anything come and find a member of staff in the mass spec lab
- For simple troubleshooting, the blue folder next to the LCMS has basic protocols for resetting the system, priming pumps etc.

Backing-up data:

- to prevent filling up the F:-or C:- drive on the main computer, data are automatically transferred to \\storage.chem.ucl.ac.uk\ms

please periodically transfer your data from this storage to your PC or your external hard drive.

DATA WILL DELETED EVERY THREE YEARS FROM \\storage.chem.ucl.ac.uk\ms and the instrument PC.