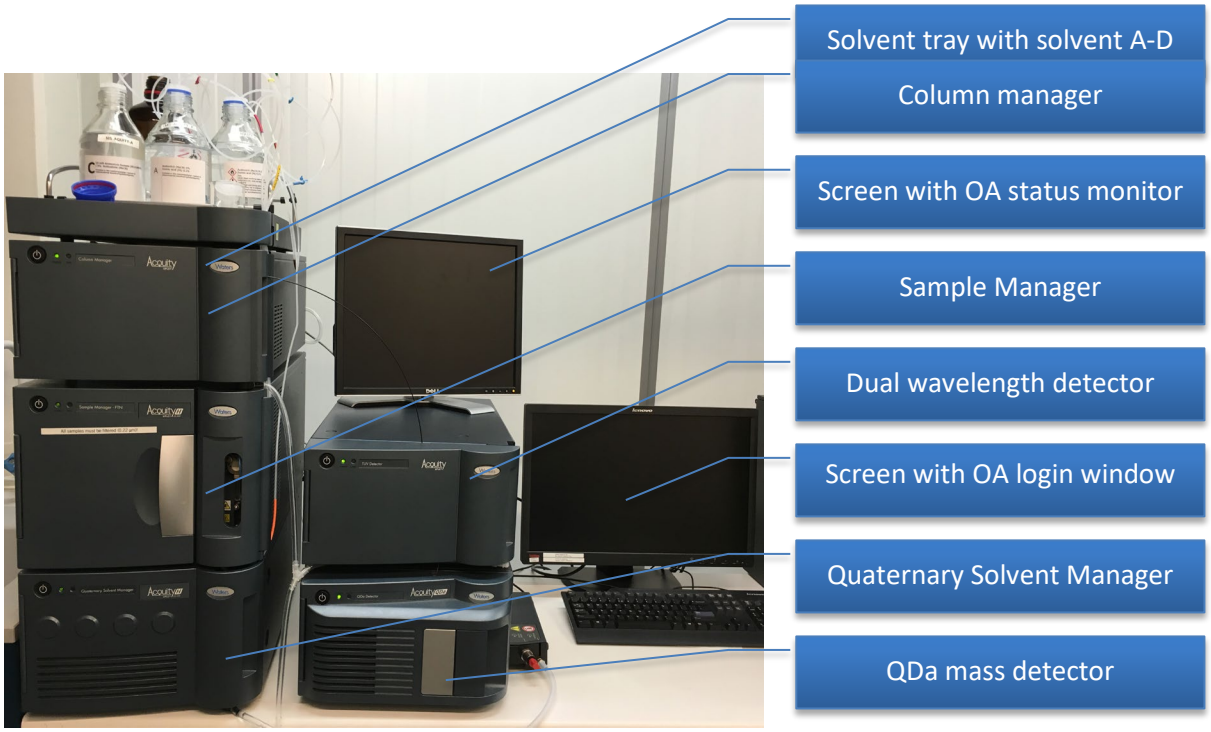
Standard Operational procedures with rules and guidelines for use of the departmental Waters Acquity UPLC-MS core facility

About this SOP

This Standard Operational Procedure (SOP) contains a short description of the instrument's organization, rules for access to and use of the instrument (sample preparation, submitting samples and retrieving data). The current version of the SOP is given in the upper right corner of the document, and the latest version will always be available at https://drug.ku.dk/core-facilities/analytical-core-facility/



The two-stack system shown above consists of a left stack comprising (from top to bottom) solvent tray with solvents A-D, a Column Manager (= column oven) with two different columns, a Sample Manager – FTN (= autosampler), and a Quaternary Solvent Manager (= pump) and a right stack comprising a TUV Dual Wavelength detector and a QDa single quadrupole mass detector.

Standard experimental for publications:
UPLC-MS analyses were performed on a Waters Acquity H-class UPLC with a Sample Manager FTN and a TUV dual wavelength detector coupled to a QDa single quadrupole analyser using electrospray ionization (ESI). UPLC separation was achieved with a C18 reversed-phase column (Acquity UPLC BEH C18, 2.1 mm × 50 mm, 1.7 µm) operated at 40 °C, using a linear gradient of the binary solvent system of buffer A (milliQ H_2O:MeCN:formic acid, 95:5:0.1 v/v%) to buffer B (MeCN:formic acid, 100:0.1 v/v%) from 0 to 100% B in 3.5 min, then 1 min at 100%B, maintaining a flow rate of 0.8 mL⁄min. Data acquisition was controlled by MassLynx ver. 4.1 and data analysis was done using Waters OpenLynx browser ver. 4.1.

Contents

Organization and user groups

The Waters Acquity UPLC-MS is part of the departmental analytical core facility. The facility is led by a steering committee (Headed by Professor Dan Staerk), and daily management is performed by the Department's NMR and MS manager in collaboration with the superuser group. The costs of running and maintaining the instrument are distributed among the users as a pay-for-use fee to section heads once per year.

Superuser group

To make sure that daily routines can be performed swiftly and securely, a group of experienced users (superusers) has been established. The superuser group currently consists of:

- ✓ Kirsten Braad Ilskov (kirsten.iskov@sund.ku.dk)
- ✓ Athanasios Papangelis (athanasios.papangelis@sund.ku.dk)
- ✓ Asmita Manandhar (asmita.manandhar@sund.ku.dk)
- ✓ Louise Kjærulff (louisek@sund.ku.dk)

Only members of the superuser group are allowed to perform maintenance, troubleshooting, changes in software settings, etc. If you are interested in becoming member of the superuser group, please talk to Louise or Professor Dan Staerk.

User group

PhD students, postdocs, lab technicians and senior academic staff can become member of the user group. By joining the user group, you also accept operating the instrument according to this SOP and follow ALL instructions given by the NMR and MS manager and/or the superuser group. To become member of the user group you will have to:

- ✓ Sign up for introduction on the door of the MS room. You will then be invited by e-mail to an introduction - within typically one week's time
- ✓ Read (and understand) this SOP
- ✓ Pass a short questioning related to the content of this SOP and a short practical introduction by one of the members of the superuser group (usually Louise Kjærulff)
- ✓ Accept that we will ask you or your supervisor to pay for repairs caused by reckless or careless operation of the instrument conflicting the rules of this SOP
- ✓ Accept that reckless or careless behavior around the instrument can lead to exclusion from the user group.

Sample preparation

The below rules for sample preparation **MUST BE FOLLOWED WITHOUT EXCEPTION**. Failure to comply with these rules will result in removal from the user group.

Dissolve the sample in a suitable solvent

The compounds must be dissolved **completely** in a 1:1-mixture of acetonitrile:water. The sample **MUST** subsequently be filtered through 0.22 µm filters for UPLC samples to avoid blockage of the small-diameter tubing in the inlet system and in-line filters. **YOU MUST FILTER THE SAMPLE EVEN THOUGH YOU DON'T SEE VISIBLE PARTICLES/PRECIPITATION**. Filtration through 0.45 µm filters for HPLC **IS NOT SUFFICIENT.**

Dissolve a little of your compound in MeCN (or MeOH), then add water to a 1:1 mixture and filter (0.22 µm) the sample in the end. Never inject solvents or reagents that will damage the column and system.

Suitable amounts and concentrations

The concentration of your sample must be below 0.1 mg/mL. The UPLC instrument with UV and mass detection is sensitive, so it is better to start with small amounts than overloading the system. Overloading can result in severe broadening of peaks, and even worse, blocking of capillaries.

If you want to analyze the progress of a reaction by monitoring the reactant(s)/product(s)-ratio, you must estimate the highest possible concentration in your reaction tube and/or calculate the dilution factor needed to **be well below a final concentration of 0.1 mg/mL in the HPLC vial**. As a guideline, the content of an open ended capillary glass tube (~10 µL) added to 1 mL of solvent (1:1-mixture of acetonitrile:water) in an HPLC-vial corresponds to a 100-fold dilution.

Sample vials and lids

All samples submitted to the Waters Acquity UPLC-MS must be prepared in 2 mL (13 x 32 mm) vials with or without insert. Regarding the cap and septum, we have had some issues with standard HPLC caps, so we currently allow only two options: Either submitting the sample without lid or using a pre-slit lid (clear lid thoroughly punched through multiple times with scissors). Thus, we do not accept samples with regular septum, plastic cap or rubber septum.

All samples must be marked with the users username on the system, e.g., MCR_KBI for Kirsten from the MCD discipline (former MCR section), and a unique sample identifier (= name of the sample). See below for examples.

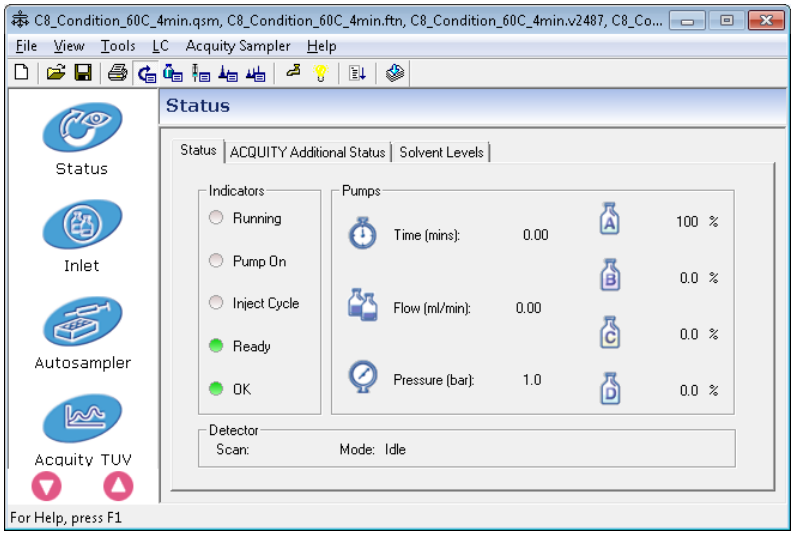
Submitting samples for acquisition

The system is set up for running open access, and instructions for this are given on the following pages. However, before submitting samples for acquisition, check that the system is ready for receiving samples and that solvent bottles contain UPLC solvent.

System readiness check

The system should be running at all times and the automation takes care of startup and shut down procedures. However, check the indicators for possible errors. The programs '`OALogin`' and '`MassLynx`' with the '`OAManager`' and '`Inlet method`' windows must be running at all times. Please, do not close any parts of the program. Contact a superuser (see page 3) if you have problems with the instrument.

- Check gas pressure on the dial by the wall to the left of the system. It should be 6-7 bars. We frequently run out of nitrogen and the MS cannot run without it.
- Check for red (blinking) lamps on the front of the machine.
- The status should usually show green indicators for '`Ready`' and '`OK`'. Other indicators might be lit during analysis of samples. When the instrument is not ready, the '`Ready`' indicator may also be red, but it should change to green shortly after you submit a sample.



Check solvents

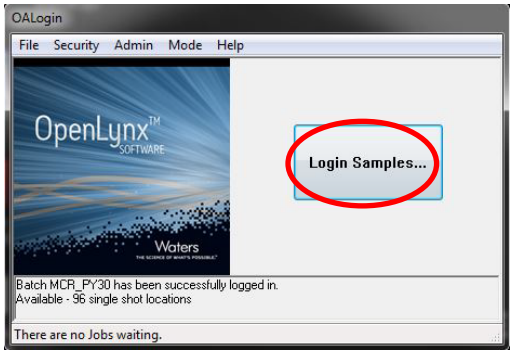
Check solvent bottles on top of the instrument. Analyses will not automatically stop if the solvent level is getting low, so please keep an eye on it and notify a superuser if it is time for a refill.

- Solvent A: 95% water + 5% acetonitrile. 0.1% formic acid
- Solvent B: 100% acetonitrile. 0.1% formic acid
- Solvent C: 95% water + 5% acetonitrile. 10 mM ammonium acetate (**not in use**)
- Solvent D: 100% acetonitrile. 10 mM ammonium acetate (**not in use**).

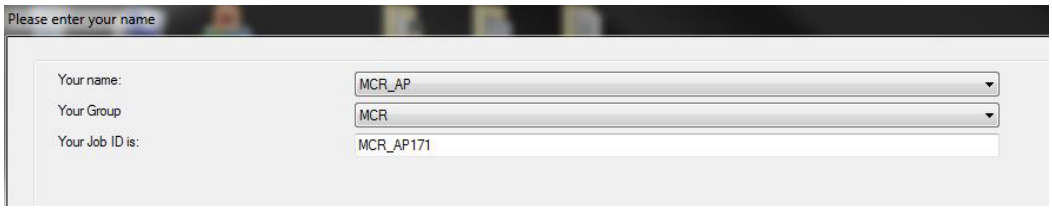
Check the waste bottles on the floor under the instrument.

Log in samples

- Press the big '`Login Samples`…'-button. If there is an error at this stage try again. If the program is busy it might reject the login.



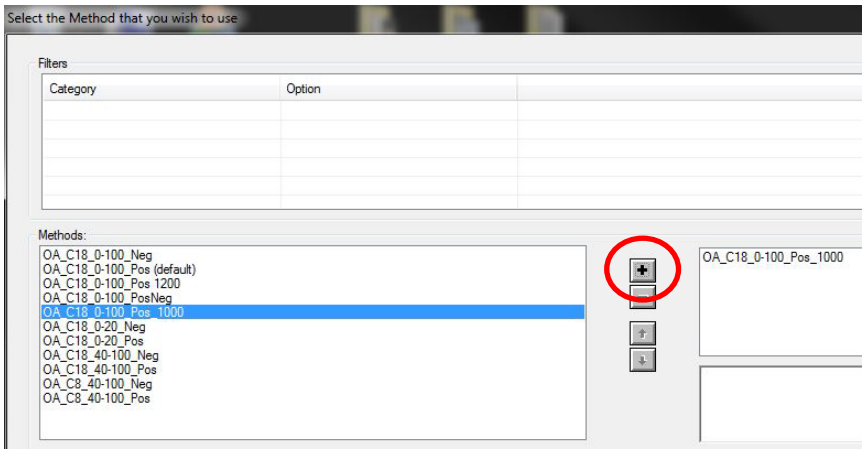
- Select your user name on the drop-down list when it appears and give a suitable name for the job ID (always starting with your user name, e.g. MCR_AP). The counter will automatically increment the number after your initials, but feel free to edit the number).



Select gradient program

Select a suitable method from the list of methods and press the '+'-button. It is possible to add several methods.

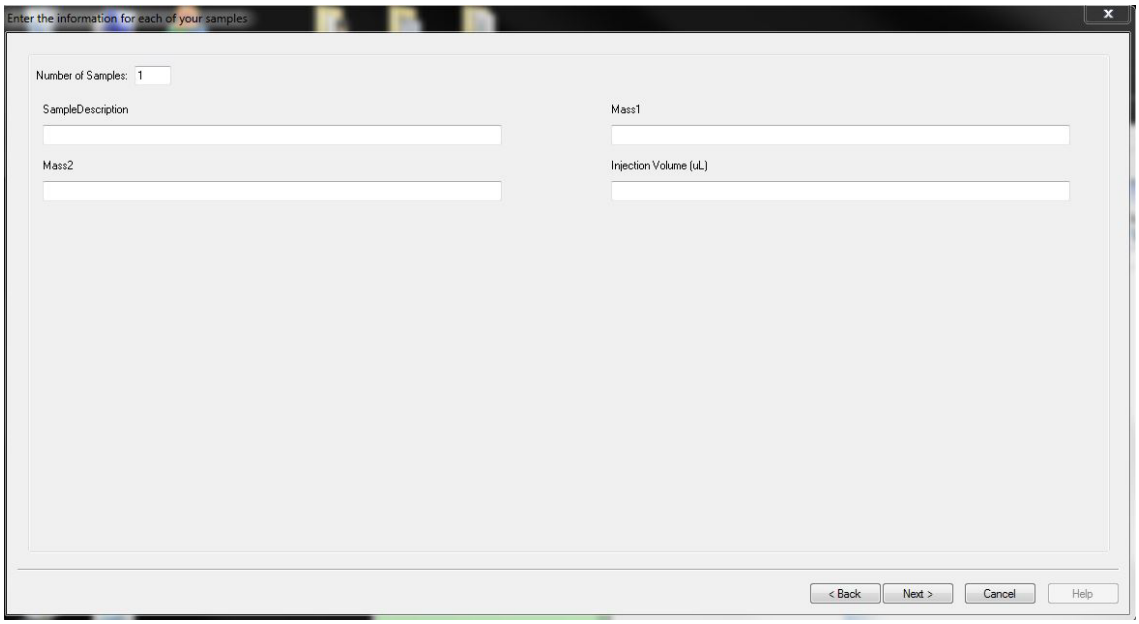
- **C18** | **C8**: The type of solid phase in the column. Choose **C18** for regular samples and **C8** for lipophilic samples.
- **0-100** | **0-20** | **40-100**: Type of gradient. Select **0-100** for a gradient from 0 to 100% B over the run-time (3.5 min to 100%, then 1 min at 100% followed by 0.5 min equilibration). Select **0-20** for polar compounds and **40-100** for lipophilic compounds.
- **Pos** | **neg**: Ionization mode. Select whether you want to use positive (ES+) or negative (ES-) mode. The most common is positive; however, certain types of compounds (e.g. carboxylic acids) ionize better in negative mode.
- **1000** or **1200**: A number in the end of the method name indicates a different mass range (1200 m/z is the highest possible).



Enter information for each of your samples

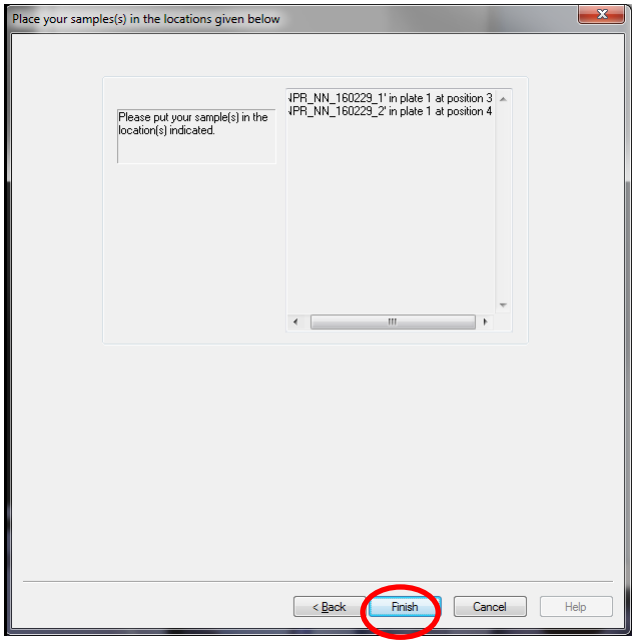
Enter number of samples (number of vials) on the top of the form. Each sample will have a page with information, and the button 'Propagate' will copy information to the next pages while incrementing the information written in the ID-field. Tip: Enter all information in the first page and then press 'Propagate'.

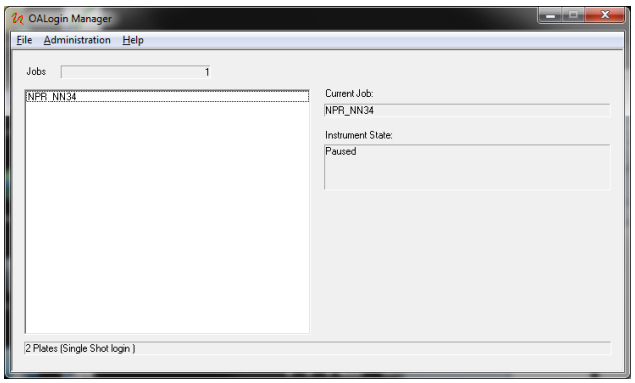
- Enter ID of the sample. This must be on the form 'Grp_User_ID' where Grp_User = your user name at the login screen and ID is (typically) your lab-journal page number and a sample number. Note the underscores. The entry in this field will be a folder-name so do not use any odd characters like ?/&%¤# or ! (a dot [.] will actually crash the system).
  Example: NPR_NN_160229_1.
- Information about the sample (will be printed on the reports) should be entered into the next field 'SampleDescription').
- Enter masses you are looking for like expected products or starting material (Mass1 and Mass2). The program will add or subtract the weight of a proton in the analysis depending on the polarity (ES+ or ES-).
- Leave the injection volume field empty. The default volume is 1 µL, which should be enough for the majority of samples. The **maximum volume is 10 µL** and higher volumes will halt the system giving an error.



Submit samples

Check that the system is not about to inject, then place the sample vials in the auto sampler compartment at the positions indicated by the next screen –pull out the sample tray and carefully slide it back in. Do not forget to **press 'Finish'**. The batch job number (your user name followed by a number) and the vial positions will be presented in the 'OA Login Manager' and in the 'OA status Manager' windows on the left-hand screen.





Data handling

- All data is saved to the C-drive as a report file (`*.rpt`, C:\MassLynx\OAlogin\Reportdb or use the 'OA DATA' shortcut) and a raw data file (*.raw, C:\MassLynx\OA Project_xxxx.PRO\Data or use the 'Data' shortcut).
- The folder LABDATA (L:\) is available for copying data from the PC to the FTP server (O:\FTP\MS\ACQUITY-A) where you can access it from an institute computer. You can also copy data via USB disk, TeamViewer or Anydesk (see info on PC screen)
- Data can be opened with Waters OpenLynx Browser (aka Diversity Browser). Instructions of how to install this program can be found on https://drug.ku.dk/core-facilities/analytical-core-facility/
- Currently there is no automatic data backup, so make sure to keep your own!