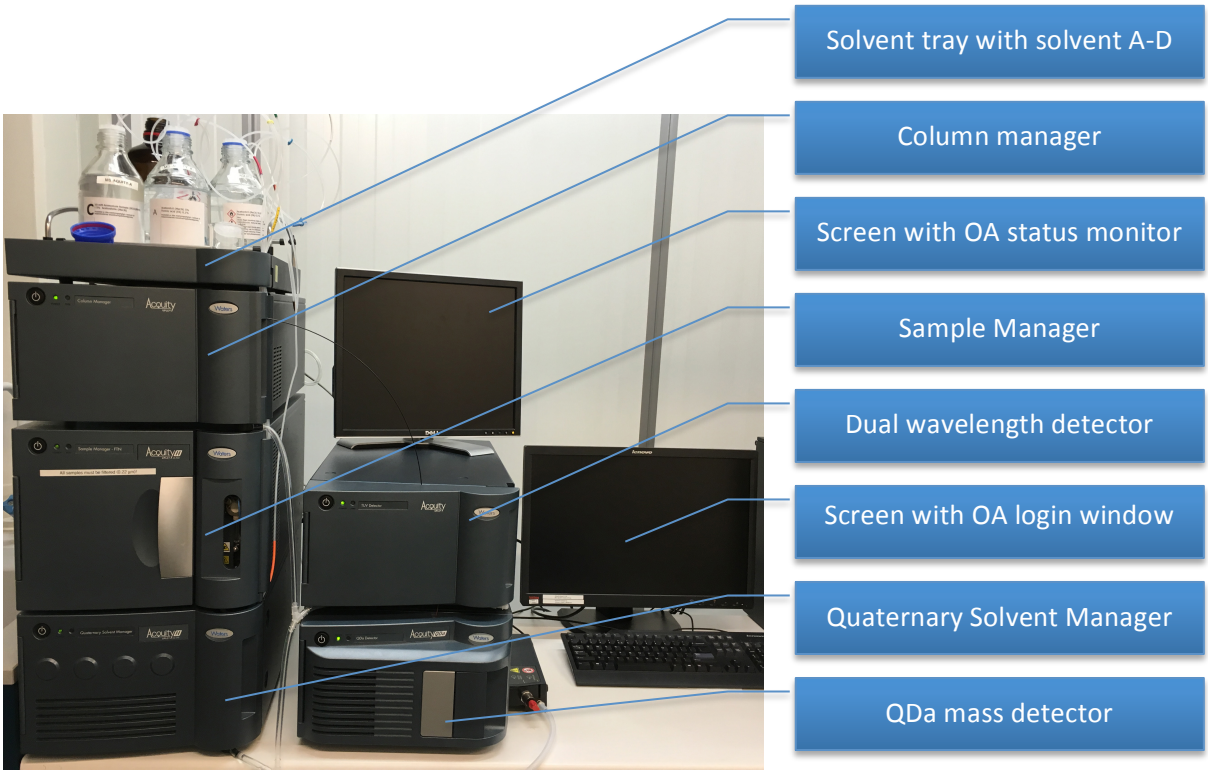
Standard Operational procedures with rules and guidelines for use of the departmental Waters Acquity UPLC-MS core facility

About this SOP

This Standard Operational Procedure (SOP) contain a short description of the instrument's organization, rules for getting access to the instrument, use of the instrument (sample preparation, submitting samples and retrieving data) and maintenance (only for the superuser group). 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 http://drug.ku.dk/research/facilities/analytical-core-facility/ms-acquity-a/.



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.

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)
- ✓ 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 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 super-user group. To become member of the user-group you will have to:

- ✓ Read (and understand) this SOP
- ✓ Pass a short questioning related to the content of this SOP by one of the members of the super-user group
- ✓ Follow a short practical introduction to use of the instrument by a member of the super-user group
- ✓ Sign or provide a signed form confirming your (or your supervisor's) willingness to pay for repair caused by reckless or careless operation of the instrument conflicting the rules in this SOP

Signing form for members of the user-group

With my signature I (and my supervisor if I do not hold an account at ILF) declare that I agree to be member of the user-group with the rules stated in this SOP. At the same time I/we accept to pay for repair caused by reckless or careless operation of the instrument conflicting the rules in this SOP.

Date: Date:

Name and signature of user Name and signature of supervisor

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 SUFFICIENTLY.**

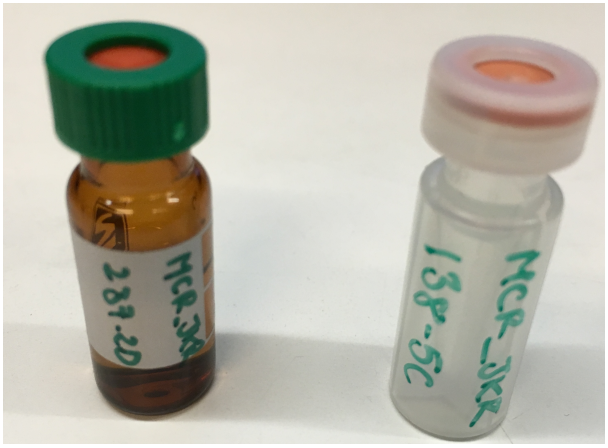
Suitable amounts and concentrations

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.

Use marked vials with caps and septum

All samples submitted to acquisition on the Waters Acquity UPLC-MS must be prepared in 2-mL (13 x 32 mm) vials with screw top, snap top or crimp top, a matching cap and a septum to minimize evaporation. All samples must be marked with the users username on the system, e.g., MCR_JKR for Jacob Krall from the MCR section, and a unique sample identifier (= name of the sample). See below for examples



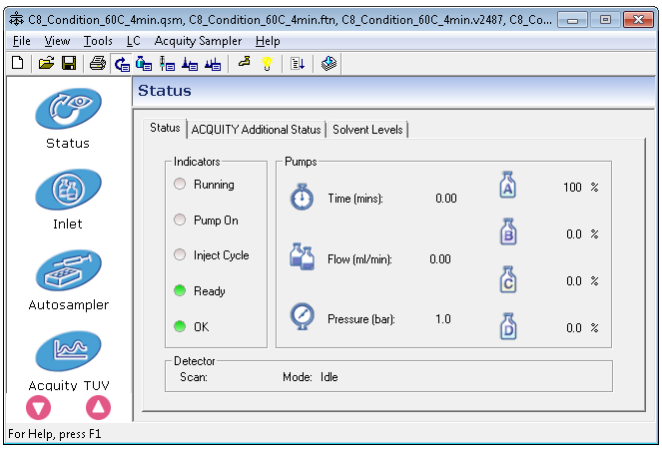
Submitting samples for acquisition

The system us set up for running open access, and instructions for this is given on the following pages. However, before submitting samples to 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`'-window must be running at all times. Please, do not close any parts of the program. Contact a Super-user (see page 4) 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.
- Check for red (blinking) lamps on the front of the machine.
- The status should show green indicators for '`Ready`' and '`Ok`'. Other indicators might be lit during analysis of samples.



Check solvents

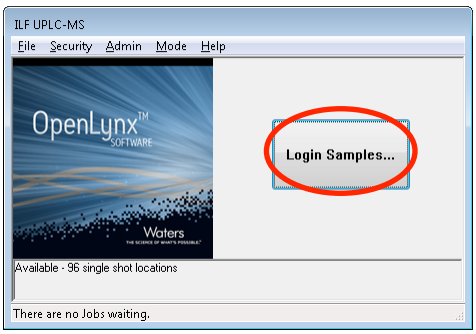
Check solvent bottles on top of the instrument. Analyses will automatically stop if the volume in the solvent bottles falls below 100 mL.

- 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.
- Solvent D: 100% acetonitrile. 10 mM ammonium acetate.

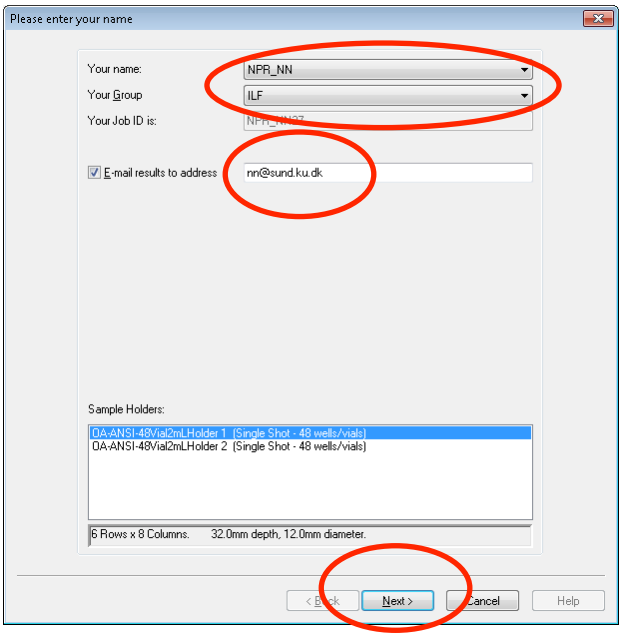
Check the waste bottles on the floor under the instrument.

Log in samples.

- Press the big 'Login Samples…'-button. Wait ~15 seconds before the next windows opens. If there is an error at this stage try again. If the program is busy it might reject the login.



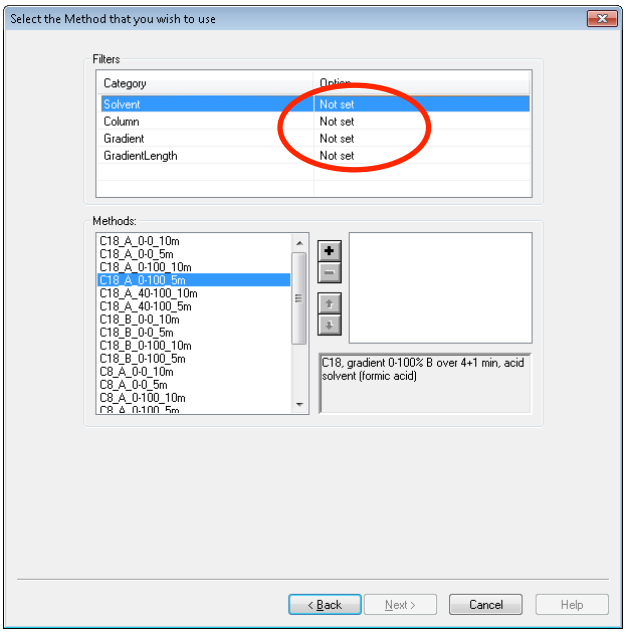
- Enter your user name on the drop-down list.
- Select group 'ILF'
- Enter e-mail address if you want the report-file to be delivered by mail.

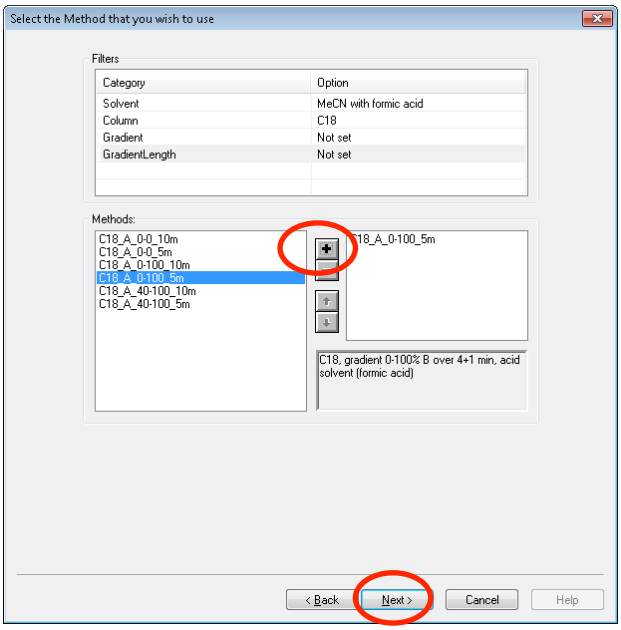


Select gradient program

- Select a suitable method from the list of methods and press the '+'-button. It is possible to add several methods.
- By selecting options in Filters, the list of methods can be reduced in length.
  - **C18** | **C8**: The type of solid phase in the column. Choose **C18** for regular samples and **C8** for lipophilic samples.
  - **A** | **B**: Acid or basic solvent system. Choose acidic (**A**) for acidic or neutral compounds and basic (**B**) for basic compounds.
  - **0-0** | **0-100** | **40-100**: Type of gradient. Select **0-0** for polar compounds – it keeps the elutropic strength at a minimum until the end of the program where a wash out is included (100% B). Select **40-100** for lipophilic compounds – it starts at 40% B. Select **0-100** for a gradient from 0 to 100% B over the run-time.

All methods use both positive and negative ionization (alternating) and use the full range of the detector – 100-1200 m/z.



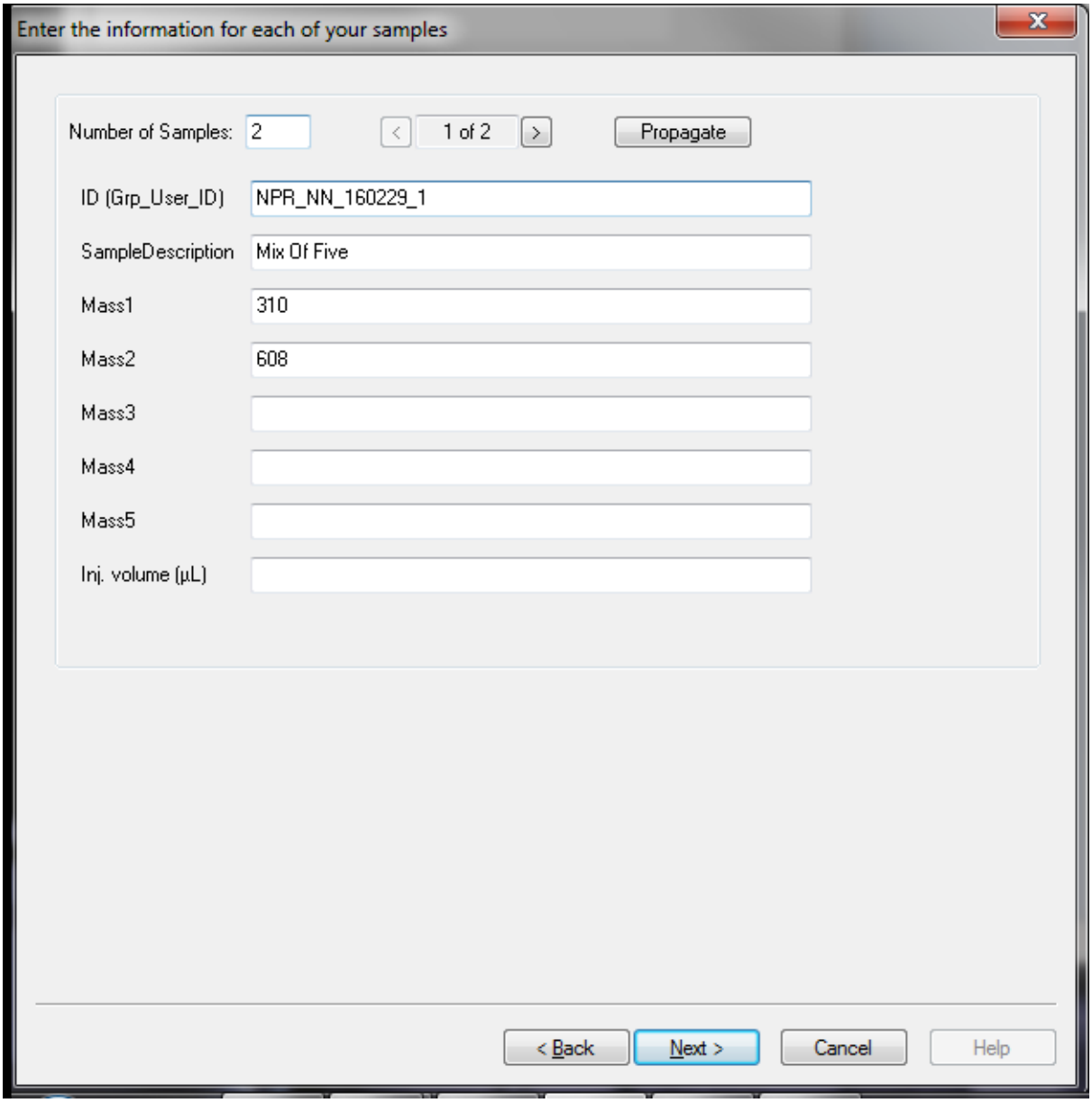


Enter information for each of your samples

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`'.

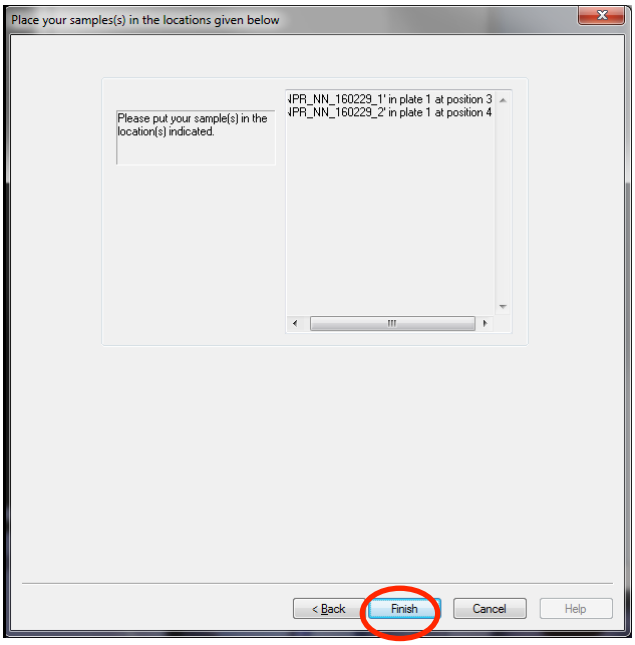
- Enter ID of the sample. This must be on the form '`Grp_User_ID`' where `Grp_User` = your user name at the login screen and `ID` is (typically) your lab-journal page number and a sample number. Note the underscores. The entry in this field will be a folder-name so do not use any odd characters like `?/&%¤#` 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`').

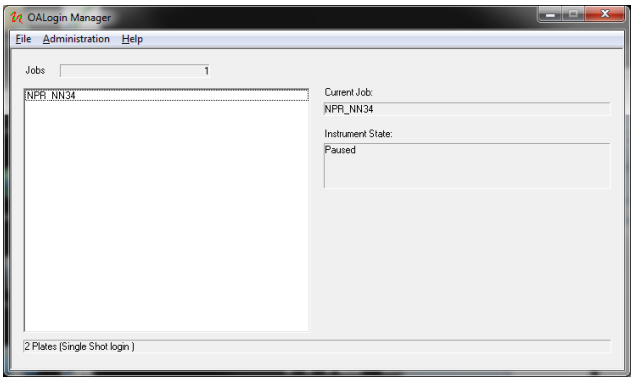
- Enter masses you are looking for like expected products or starting material (`Mass1` – `Mass5`). The program will add or subtract the weight of a proton in the analysis depending on the polarity (ES+ or ES-).
- Leave the injection volume field empty. The default volume is 1 µL, which should be enough for the majority of samples. The **maximum volume is 10 µL** and higher volumes will halt the system giving an error.

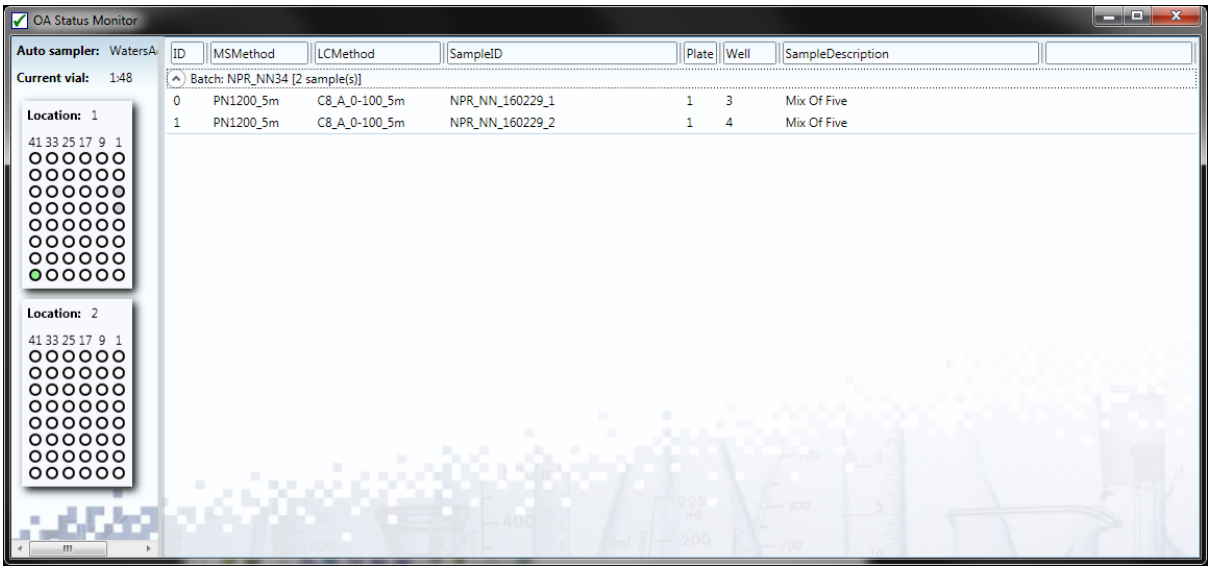


Submit samples

Place the sample vials in the auto sampler compartment at the positions indicated by the next screen. 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 are saved to the E-drive and this drive is backed up every night.
- Recent raw data is saved in the folder "`E:\MassLynx_Projects\ILF_20xxxx.PRO\Data`". Report files (`*.rpt`) and print outs (`*.pdf`) are initially saved in the folder "`E:\Reports` ".
- Report and pdf's are also copied to the ILF-network drive "`O:\FTP\MS\MS-ACQUITY-A\Reports`" where it is accessible for three months before they are automatically removed.
- Report files (`*.rpt`) are also sent by mail. These can be opened with Waters OpenLynx Browser (aka Diversity Browser). Instructions of how to install this program can be found on http://drug.ku.dk/research/facilities/analytical-core-facility/software.
- Reports and printouts are moved automatically (by a script run every night) to the folder "`E:\OldReports`" where it is saved and organized both per user and per date.
- Data are not deleted from the workstation and backed up at regular intervals. If you really (really, really, …) want your data to be safe, the best procedure is to make your own backup to a USB-disc.