User manual and instructions for Agilent 6530 UHPLC-QTOF

with Agilent 1290 UHPLC

Contents

1. Contact information

In case of assistance or support needs with the instrument contact primarily to Staff scientist Anniina Kiesilä. For needs with permissions, data interpretation or method development, please contact primarily Lab manager Elina Kalenius. If you notice shortage in N2 collision gas, please inform Lab manager or staff scientist or contact directly Hannu Salo.

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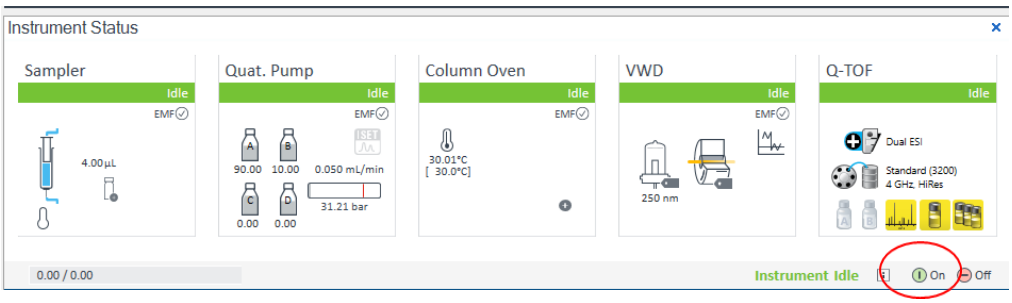
2. Sample preparation

For sample preparation use the balance and solvents found in MS laboratory (O309) (instructions to use balance are found in MS lab General Instructions document). Weigh an exact amount of sample and prepare a 1-5 mM stock solution. Then dilute the sample to appropriate concentration, recommendation is to start with 50 µM concentration. The samples can be prepared to MeCN or H_2O. Last and most importantly, **filter all the samples through 0.2 µm filter** to remove any solids which might block the analytical column used. For samples in H_2O syringe filters can be used, for organic solvents use centrifuge filters. Both filters and syringes can be found in MS laboratory.

Note that with 6530 instrument it is allowed to measure organic compounds only! Metal complexes and other metal containing compounds should be measured with other instruments. If you are unsure about your compound, please contact lab manager or staff scientist.

3. Opening the software

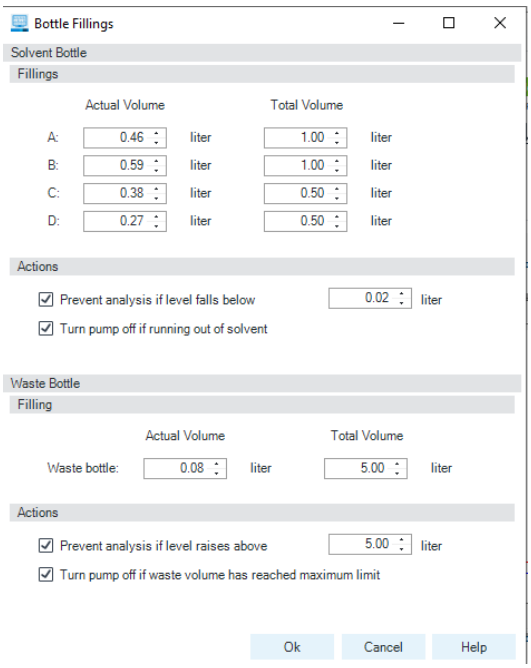
Make sure that all the compartments are shown in green in the Instrument status slide. If not, put the instrument on by pressing *on* in Instrument status. In case any of the compartments are shown in red (error) contact staff scientist or lab manager.



4. Logbook

Fill the logbook during the measurements. Before starting the measurements, check the gases and mark their pressures into the logbook. The CID N_2 bottle should have more than 20 bar and nitrogen generator should give 80-100 psi for normal operating pressure. If you notice that the gas pressure is not sufficient for your measurements, inform the staff scientist or manager.

5. Solvent lines



Normal user:

Before starting, plan that which solvents you are going to use

Line A: H_2O

Line B: MeCN

Line C: H_2O + 0.1% TFA (Trifluoroacetic acid)

Line D: H_2O + 0.1% FA (Formic acid)

Check that solvent lines have enough solvent for your runs. **Check the seal wash and flush port bottles**, both must have > 50 ml of solvent. If the needed solvent levels are low for your runs, ask MS lab personnel to fill them.

Instrument has a constant flow from lines A and B, but if you will take into use line C or D, you need to **purge the line C or D** before use. Right click on top of Quat Pump icon → Prepare pump. Choose the line that you wish to purge by changing its composition to 100% , then purge that line 5 minutes with the rate of 5 ml/min by pressing start.

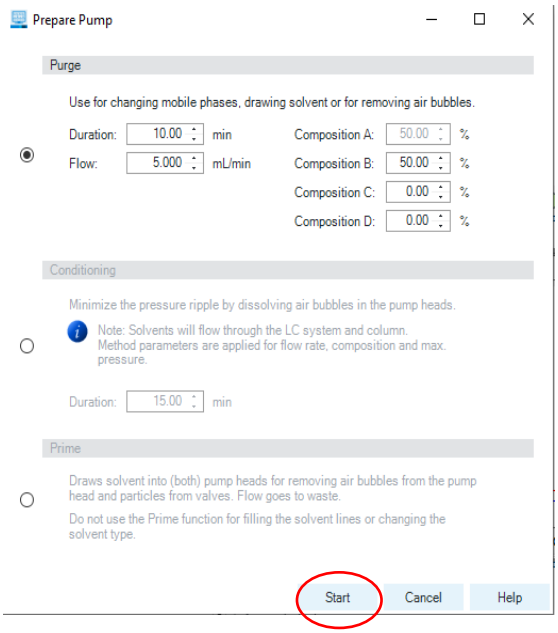
Advanced user:

Check the solvents for UHPLC in lines A-D. Estimate the solvent consumption of your runs and fill the bottles if needed. Sonicate all added solvents.

Record the solvent level to the software in *bottle fillings*. Right click on top of Quat Pump icon → Bottle fillings. The pump will turn off automatically if the solvent bottle filling information gets below 20 ml. Remember to leave at least ~100ml of solvent to line A at the end of the measurements! (~250ml over the weekend).

Solvents should be changed if instrument has been long time unused or solvents are older than 1 month.

Check the seal wash and flush port bottles. If bottle has < 50 ml left, prepare a new one. Seal wash: H_2O / IPA 9:1, Flush port: H_2O /MeCN 50:50.



Check the waste bottle under the table. If the waste can is almost full, replace it with new one. Remember to update this action in *bottle fillings*! The pump will automatically turn off if waste bottle gets full.

Purge the lines you are going to use from *Prepare pump*. Right click on top of Quat Pump icon → Prepare pump. Choose the lines that you wish to purge, then purge each line at least 5 minutes with the rate of 5 ml/min.

To prepare a new eluent:

Take a new solvent bottle. Rinse the bottle few times with small amount of solvent that you are going to use as an eluent. Add the solvent and mark the date and solvent to the bottle. Sonicate the eluent ~10 minutes prior to use to homogenize it and to remove air bubbles.

To replace an eluent:

If you are going to replace the bottle with the same solvent, remove the sinter from the old bottle and insert it directly into the new one. If you are going to change the eluent to a different one (also if the old one had any additive, and new one does not!), then rinse the sinter in a 50 ml beaker filled with the new eluent and start the purge from beaker before inserting it into the new bottle. Discard the old eluent in the fume hood, wash the bottle and leave it dry.

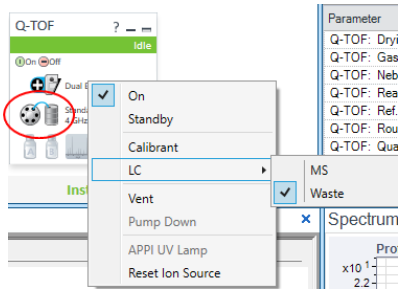
To prepare a buffer:

Prepare the buffer and adjust the pH. Check column specifications to make sure that the column can be operated in that pH! Filter the buffer through 0.25µm filter paper (whatman 42) and sonicate the buffer 15 – 30 minutes prior to use.

6. Equilibration of the column

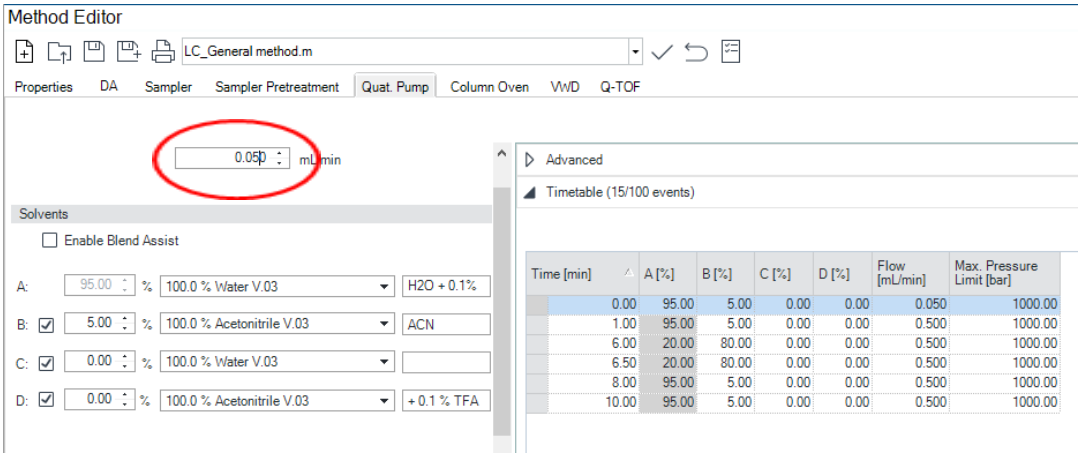
The stand by flow to the instrument is 95:5 A:B (H_2O / MeCN) with 0.05 ml/min flowrate. Check that the LC flow goes to the waste and calibrant is off.

Right click on top of QTOF icon → LC flow → waste.



Start increasing the flow gradually (Method editor → Quat pump → flow rate) 0.1ml/min at the time until you reach the target flow rate, for example 0.5 ml/min if you are using one of the General methods. Each time you change the flowrate, press *apply* to send information to instrument. Follow the pressure of the column constantly. The maximum flowrate is 0.5 ml/min. Finally, open the method you want to use in the runs, for example General method or General method_TFA or _FA. Let the column stabilize at least 10-15 minutes before starting the measurements.

If you need to change the eluent ratio, change it gradually 5-10% at the time. Follow the pressure. If you do any changes to the methods, save them with your name to your own folder.



7. Generating new method

The general method uses: H_2O / MeCN (lines A and B)

General method_TFA: H_2O + 0.1% TFA / MeCN (lines B and C)

and General method_FA: H_2O + 0.1% FA / MeCN (lines B and D)

All methods have a similar gradient and parameters

Time (min)	H_2O (+ acid) %	MeCN %
0	95	5
1	95	5
6	20	80
6.5	20	80
8	95	5
10	95	5

The flow rate is 0.5 ml/min, run time 10 minutes, no post time, injection volume 5 µl, needle wash from flush port (15 s with 3 reps), column oven 30 ° C. Ion source parameters are the following:

Gas Temp 325 °C, Drying gas 10 l/min, Nebulizer 20 psi, Sheath gas Temp 325 °C, Sheath gas flow 11 l/min, VCap 4000 V, Nozzle 2000 V, Frag 200 V, Skimmer 60 V, Oct 1 RF Vpp 750 V.

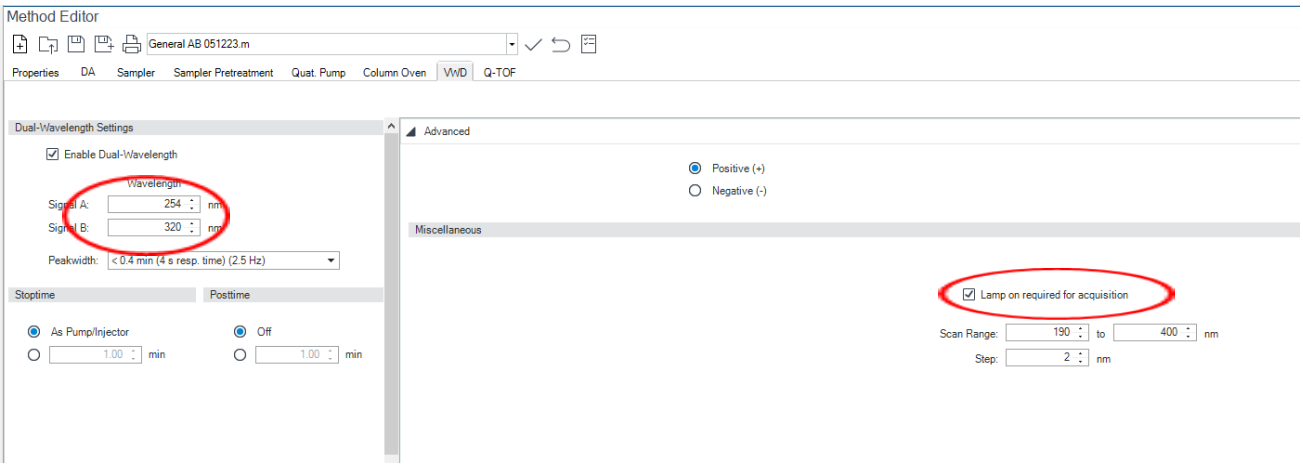
(+)ESI-MS, *m/z* 50-2000

You can build your own method using one of the methods as a starting point. Remember, that **if you do any changes to these methods, save the modified method as a new method** with your own name to your own folder!

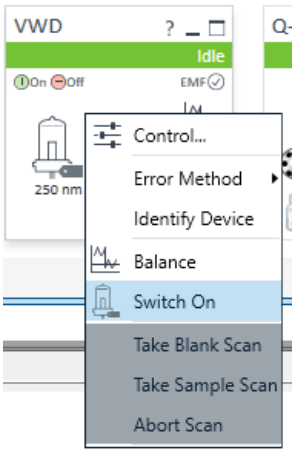
8. Use of UV detector

If you are not using UV detection, move to the next chapter.

If you want to also measure UV, choose the VWD from method editor. Choose the wavelength(s) you want to acquire. Tick Lamp on required for acquisition. Note that general method does not have UV chosen, and you will have to save the modified method with your own name to your folder!



After this turn the UV lamp on from VWD. Right click on top of the icon, and choose switch on. The UV-lamp needs to warm up for ~15-20 minutes before the runs.

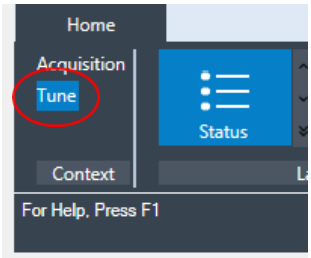


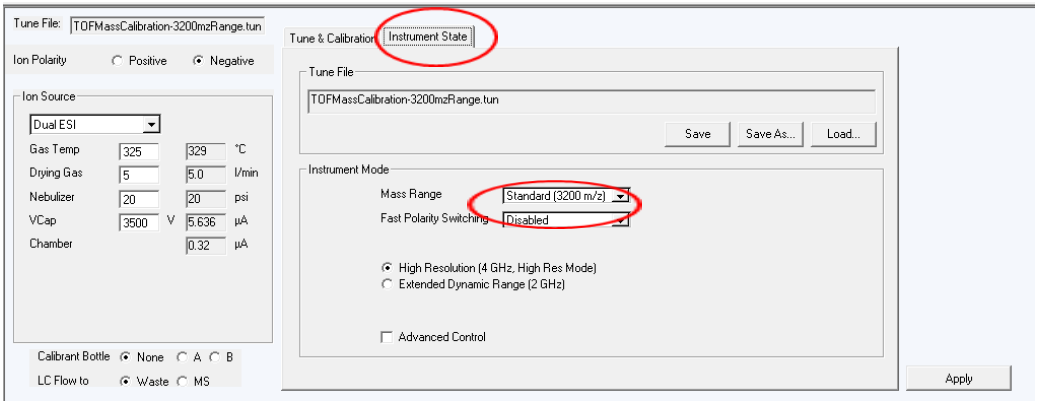
9. Calibrating the instrument

Check that Calibrant B bottle has calibrant. If calibrant runs out, inform the Staff scientist.

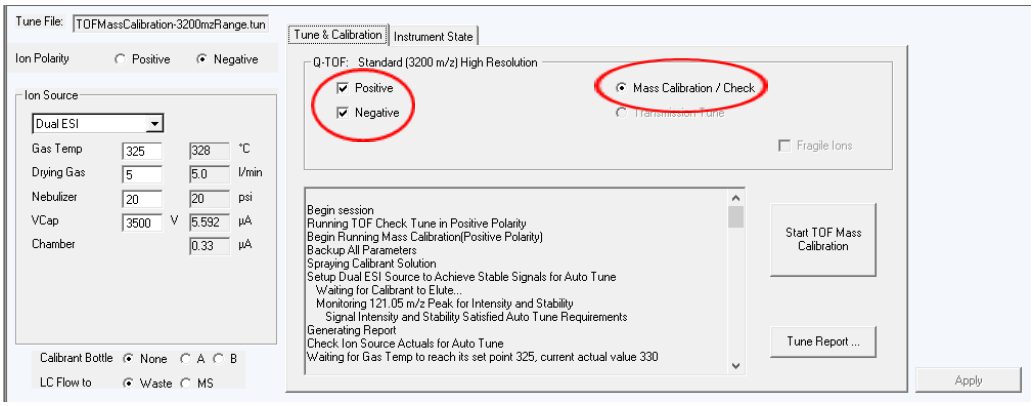
From *Acquisition* context move to *Tune* (up in the left corner).

From *Instrument state*, choose the Standard 3200 *m/z* mass range. Fast polarity switching should be disabled and High resolution chosen.





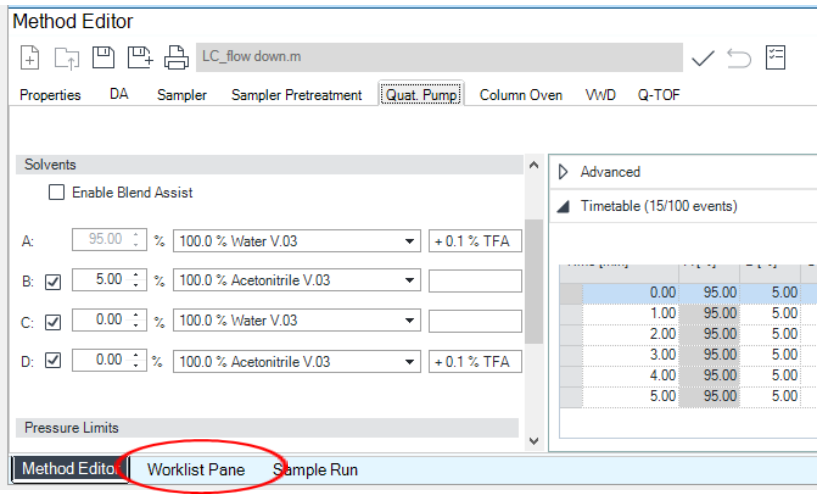
In *Tune & Calibration*, choose the polarities where you want to calibrate (positive, negative or both) choose Mass Calibration/Check and press Start TOF Mass Calibration. After calibration Tune reports are displayed.



9. Acquisition of the data

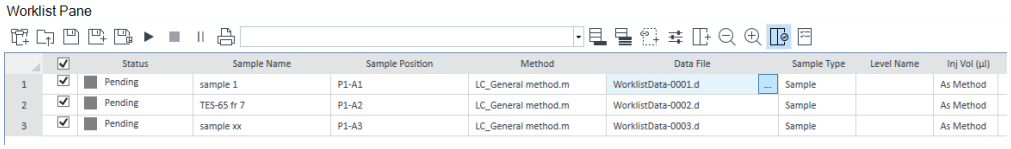
When calibration is finished, move back to *Acquisition* context. Now the LC flow starts flow directly to the MS (if it does not, switch it by right click QTOF icon and change the LC flow from waste to MS).

From Method editor, move to the Worklist Pane.



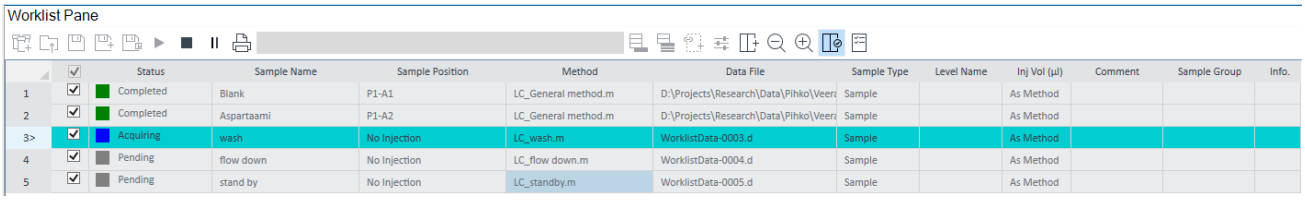
In Worklist, create a new list (or open your former modified list) and add your sample(s) to the list. Place the vials to the multisampler and add the locations to the list accordingly. The locations have to be in form P1-A1 (P1 = plate 1 or 2, A1 = coordinate of your sample in the plate). If there are old samples inside multisampler, place them to measured MS samples tray on the table.

Choose the method you want to use for each sample. In data file, choose your folder as location! Create a new folder to your data folder and name it YEARMMDD_yourname. Save all your data to this folder. Note that you must name all your data files with different name or number. If you are measuring multiple samples, and want the instrument to go standby after them, add the end methods at the end of the worklist (see next chapter). If there are reservations after you, you must run the end methods before the next user! The duration of the end methods is 25 minutes in total. When the worklist is ready, or just to run a single sample, choose the correct row(s) you want to run, and press run worklist.



10. Ending the experiments

After your last sample, or at the end of the worklist, add three more samples. For all samples choose “no injection” as sample position. For first row, choose method LC_wash. For second, choose the method LC_Flow down. For third use the method LC_Standby. If you have already started to run your worklist, you can press pause button on top of the worklist. The worklist is then paused after the current run, and you can add the methods to the queue. Then run the whole worklist. Pick up your samples after all the runs are finished or on the following day. Inform the Staff Scientist if you need longer storage for the samples, normally they will be stored only few days.

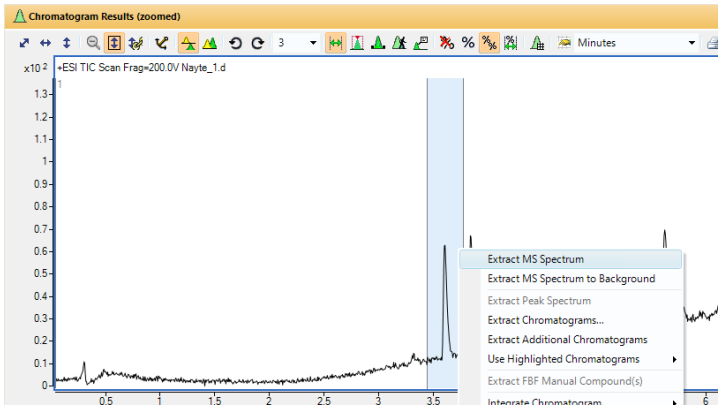


11. Data analysis

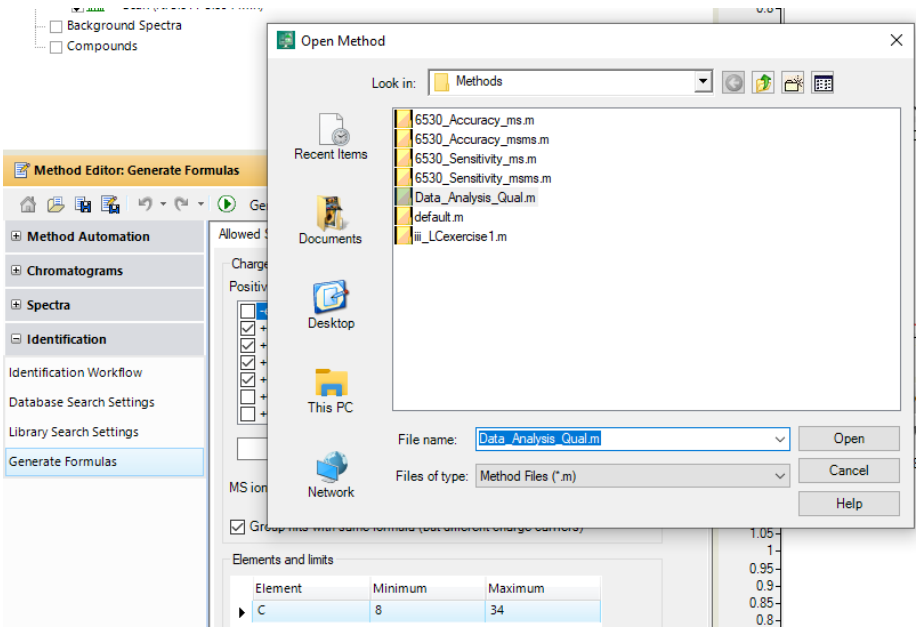
Data analysis can be done either in laboratory or using remote Data-PC (please see the separate Data-PC instructions).

Open Qualitative Analysis B.09.00.

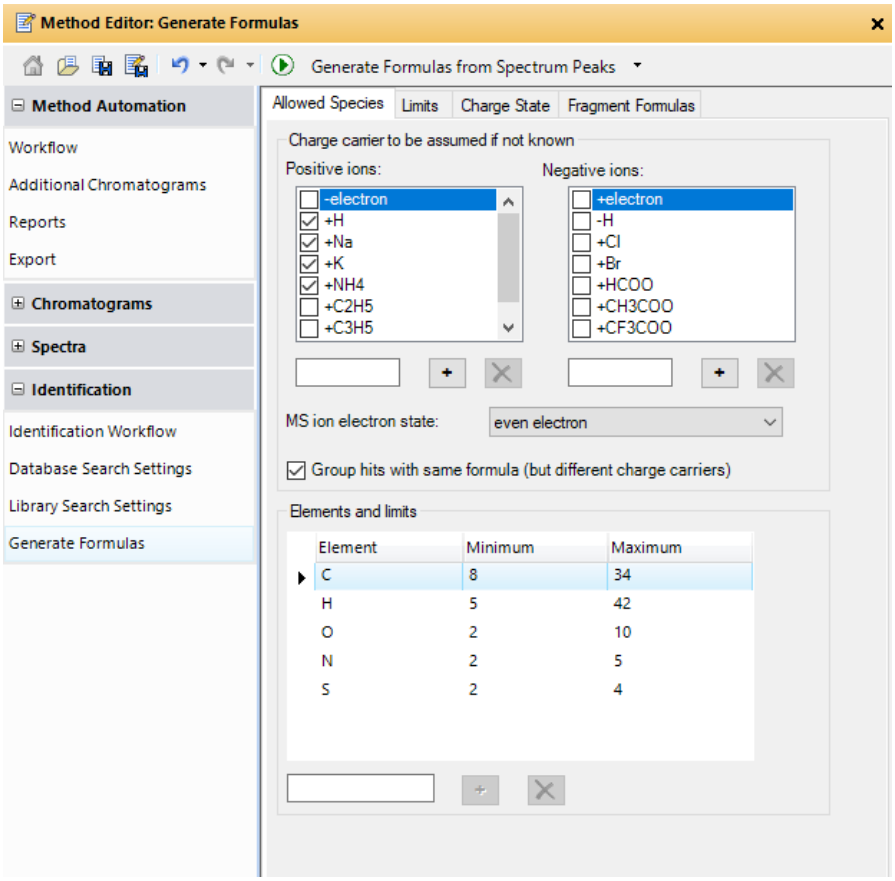
Open your data file. With mouse left button, choose the area that you want to extract from the chromatogram, and press right button of the mouse → Extract MS Spectrum.



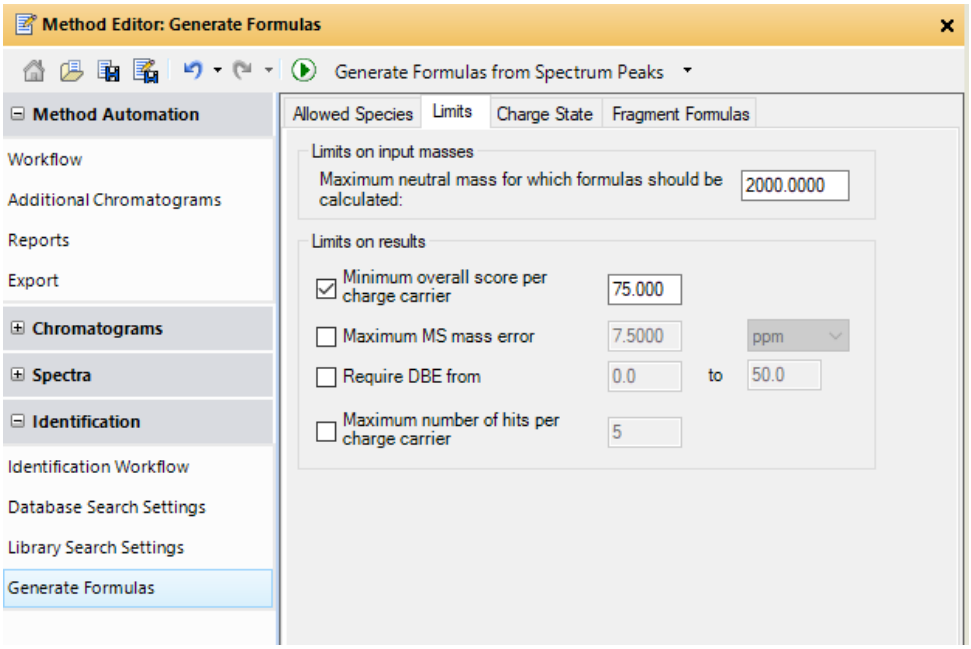
Analyse the spectrum. If you observe your compound, you can generate the accurate mass report. To get the report for accurate mass, open from the method editor (down on the left) Data_Analysis_Qual method.



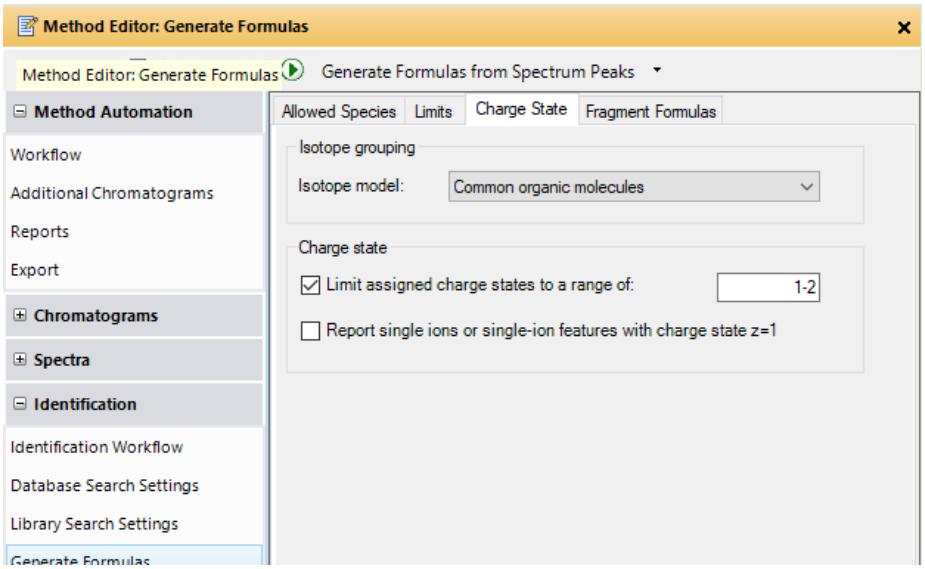
From Identification → Generate formulas → Allowed species: choose the limits for your target compound and the ions it could form (negative, positive, how you expect it to ionize).



In Limits, determine the maximum molecular weight for your compound.

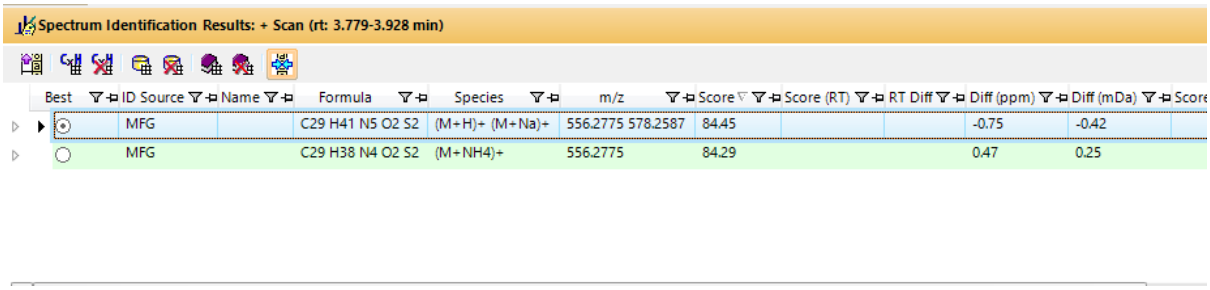


In charge state, choose the appropriate Isotope model and charge states.

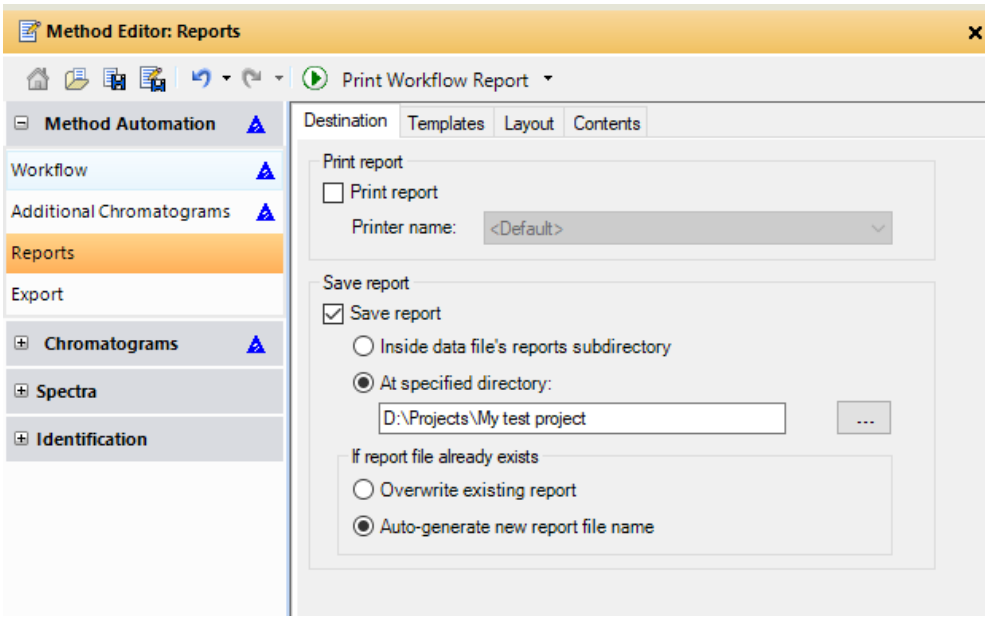


Make sure the correct spectrum is chosen active, and press Generate Formulas from Spectrum Peaks.

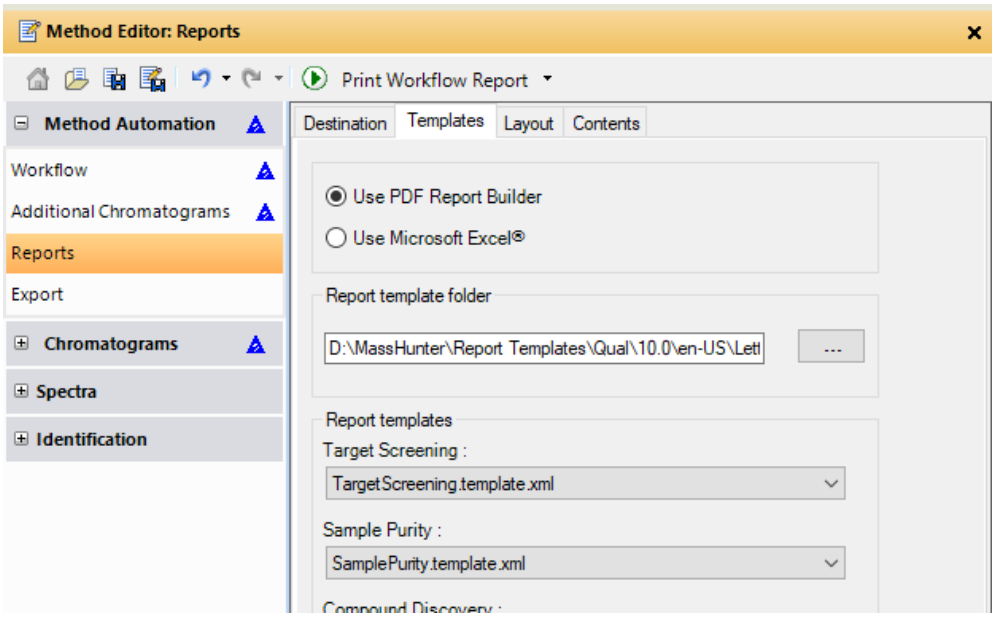
Spectrum Identification Results appear. Choose the one matching to your compound.



In Method Automation → Reports → Destination → Save report → At specified directory and navigate to your own folder!



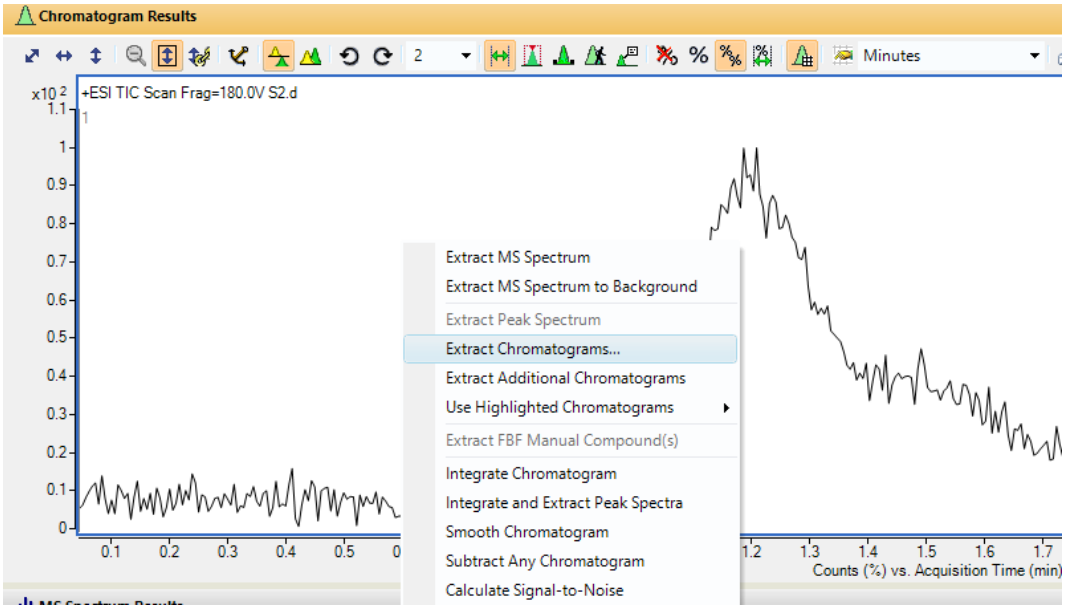
In templates, check that you have Qual\10.0\en-US\Letter chosen as shown in the figure. Press Print Workflow Report.



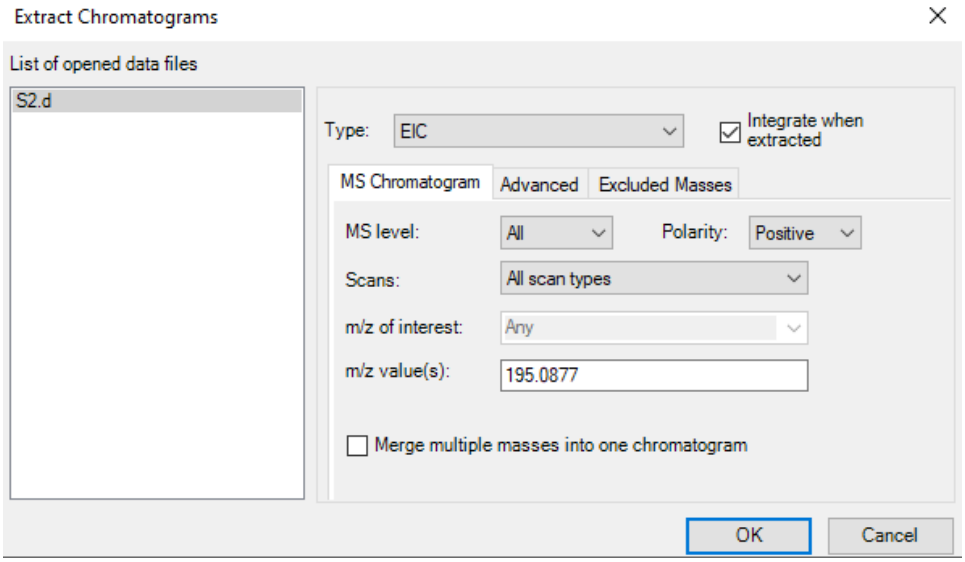
Cannot find the compound from chromatogram?

Calculate theoretical *m/z* value for the ion, for example by using Isotope distribution calculator with icon:

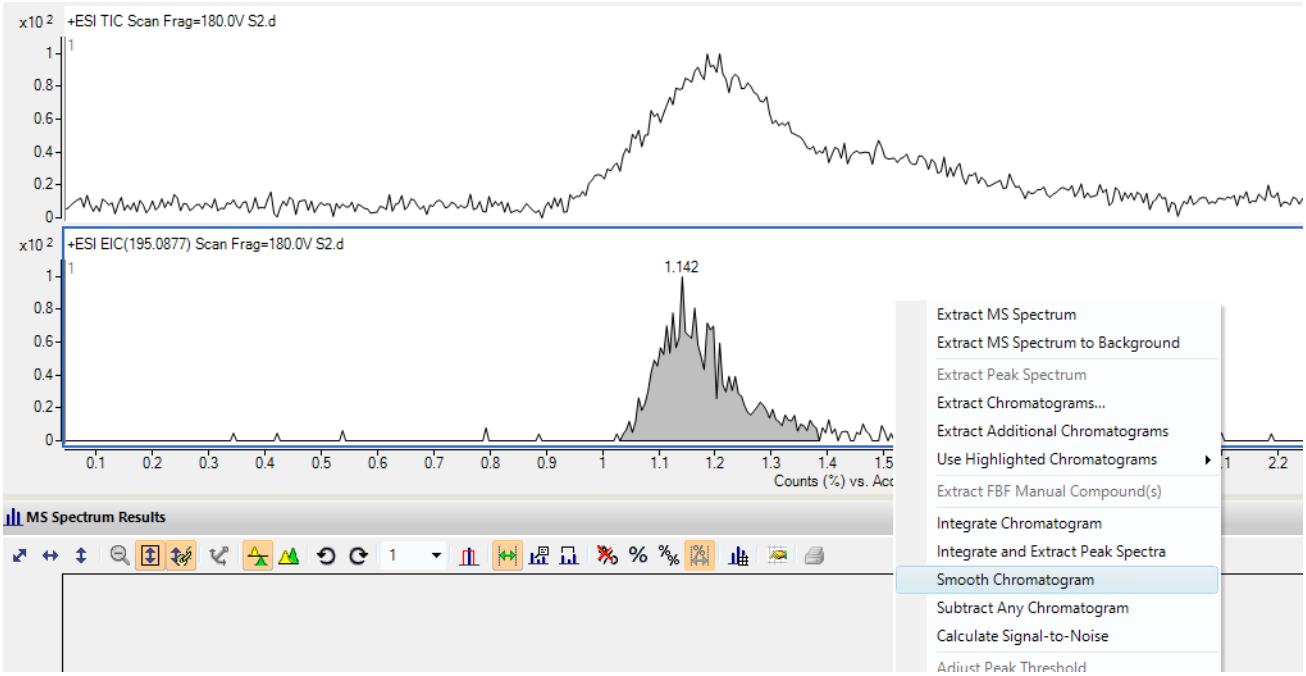
Right click the mouse on top of chromatogram, and choose Extract chromatograms.



Choose EIC as Type. Write the accurate *m/z* value you want to extract from the chromatogram and press OK.



If an ion with similar *m/z* is found, you will see its retention time in extracted ion chromatogram. Right click on top of the chromatogram and choose Smooth chromatogram. Then from smoothed chromatogram extract the MS spectrum and see if your compound can be found in the spectrum.

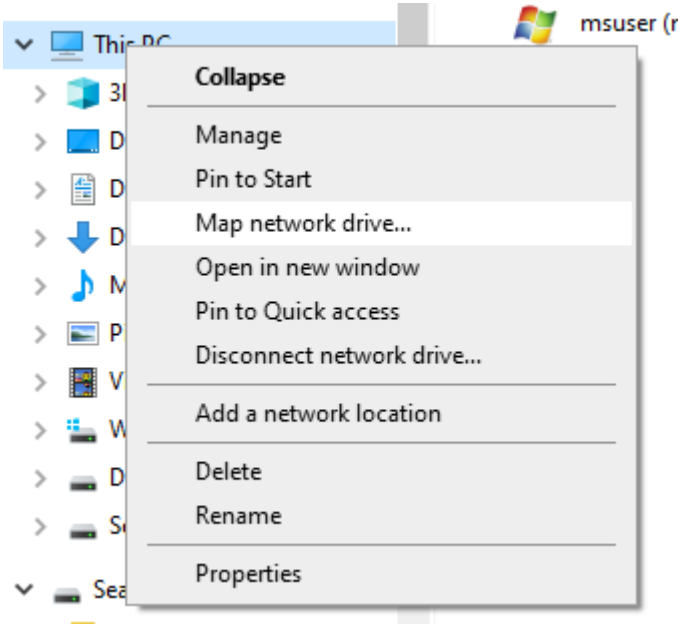


12. Use of S-Drive

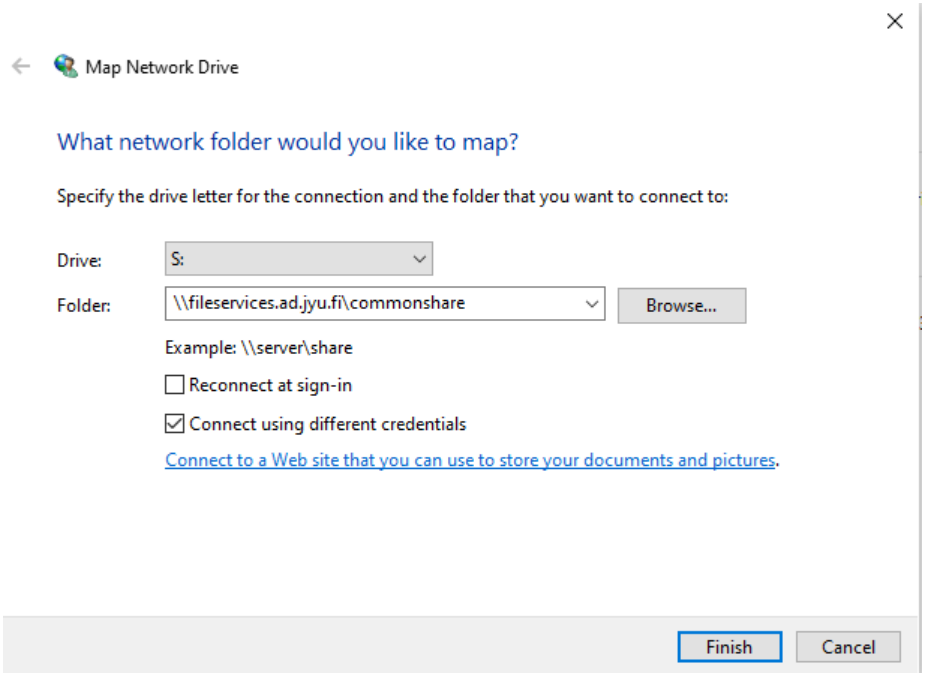
If you need more time to analyse your data, please move your data to S-drive and book the Data-PC from infrabooking. The same softwares for data analysis are available on Data-PC. See also instructions for the use of Data-PC, which can be used remotely.

To transfer your data to S-drive:

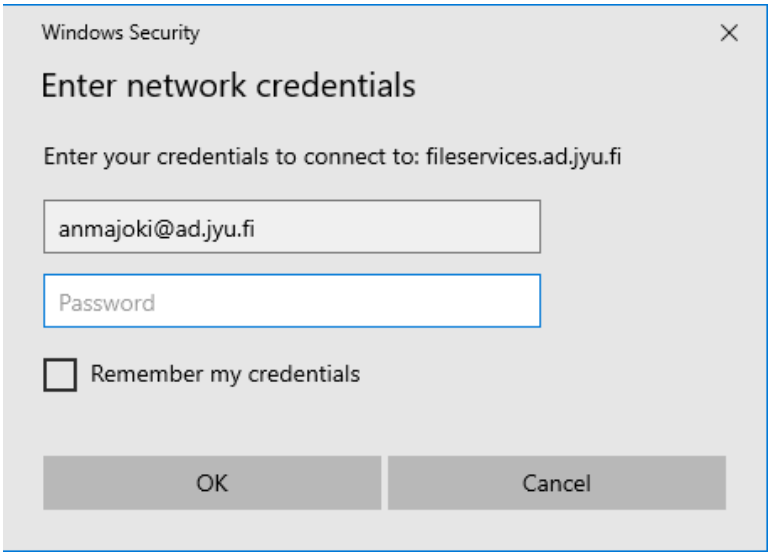
On top of This PC, press mouse right and choose Map network drive.



On Map Network Drive, choose the following options and press Finish.

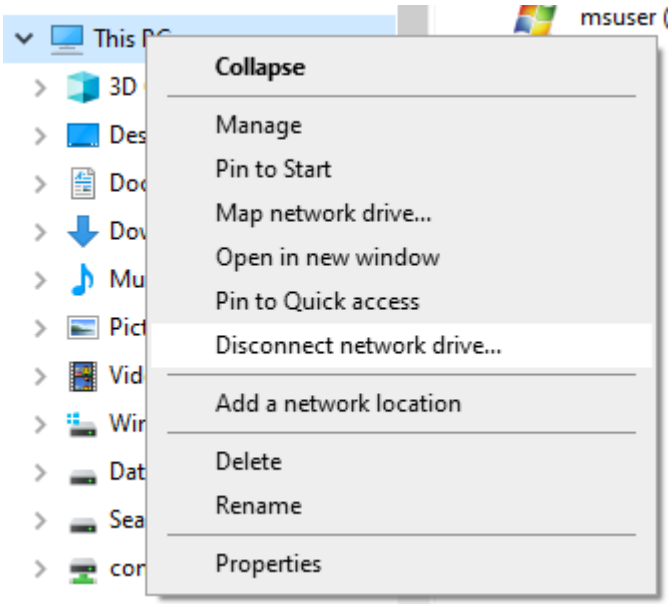


Write your username and password. Note: the username has to be in the form username@ad.jyu.fi



After pressing OK the connection to S-drive opens. When you have transferred your data to S-drive, take care to disconnect the S-drive!

On top of This PC, press mouse right and choose Disconnect network drive.



Select the network drive, and press OK.