

User guide for operating the Agilent 6420A QqQ mass spectrometer by direct infusion/injection

This user guide does not have the intention to introduce you to any of the theory and/or principles behind triple quadrupole mass spectrometry. All aspects of ionisation, collision (in source, CID etc), parameter-ion relationships, reaction monitoring/acquisition (SRM, MRM, MS2scan, MS2Sim, neutral gain, neutral loss), spectra processing, qualitative and quantitative data treatment, should be learned elsewhere or on your own. The document should only make you capable of operating the instrument and control software. If then your choice of experimental settings/conditions is appropriate for your target sample you will obtain a mass spectrum.

The instrument is an Agilent 6420A triple quadrupole (QqQ configuration) mass analyser using electrospray ionisation (ESI). It is connected to an Agilent 1200 series LC module (binary pump, column compartment/oven and auto sampler).

Samples can be introduced to the spray chamber either through the LC column or by flow injection (no LC column).

The LC column primarily installed on the instrument is an Agilent ZORBAX SB-C18, RRHT; 2.1 x 50 mm, 1.8 µm.

A mass spectrometer is very sensitive towards contamination and concern has to be taken regarding sample preparation and concentration of samples that are injected into the instrument. To prevent contamination of your sample and the instrument you should *always*;

- Handle and prepare samples correctly.
- Use clean glassware when preparing dilutions and final samples (rinse with high quality solvent prior use).
- Use solvents of high quality.
- Use clean and certified vials (rinse with high quality solvent prior use).
- Cap septum (for auto sampler vial) must be resistant for the solvent used. You can avoid using cap if not strictly necessary.
- Avoid using Eppendorftm pipettes for organic solvents.
- Filter the final solution to remove particles. Minimum through a pore size of 0.45 µm (purge the filter with high quality solvents before sample filtration). *Recommended: Chromacol 4-SF-45(N) supplied by Teknolab. Available in the department store.*
- Avoid toughing vial septum with bare fingers.

Concentrations:

Direct infusion full scan (MS2Scan) acquisition (scan range ~500 u); 2-10 µg/mL (for compounds around 300-400 g/mol). For narrow scan ranges (typically 100 u, sample concentration has to be reduced)

Direct infusion MS2Sim, SRM, MRM acquisition; 100-200 ng/mL/component (for compounds around 300-400 g/mol)

When using the instrument you should always fill out the log book.

When you leave the laboratory, collect your equipment, clean the hood (if used) and remove your vials from the auto sampler.

Lock the door when leaving the laboratory.

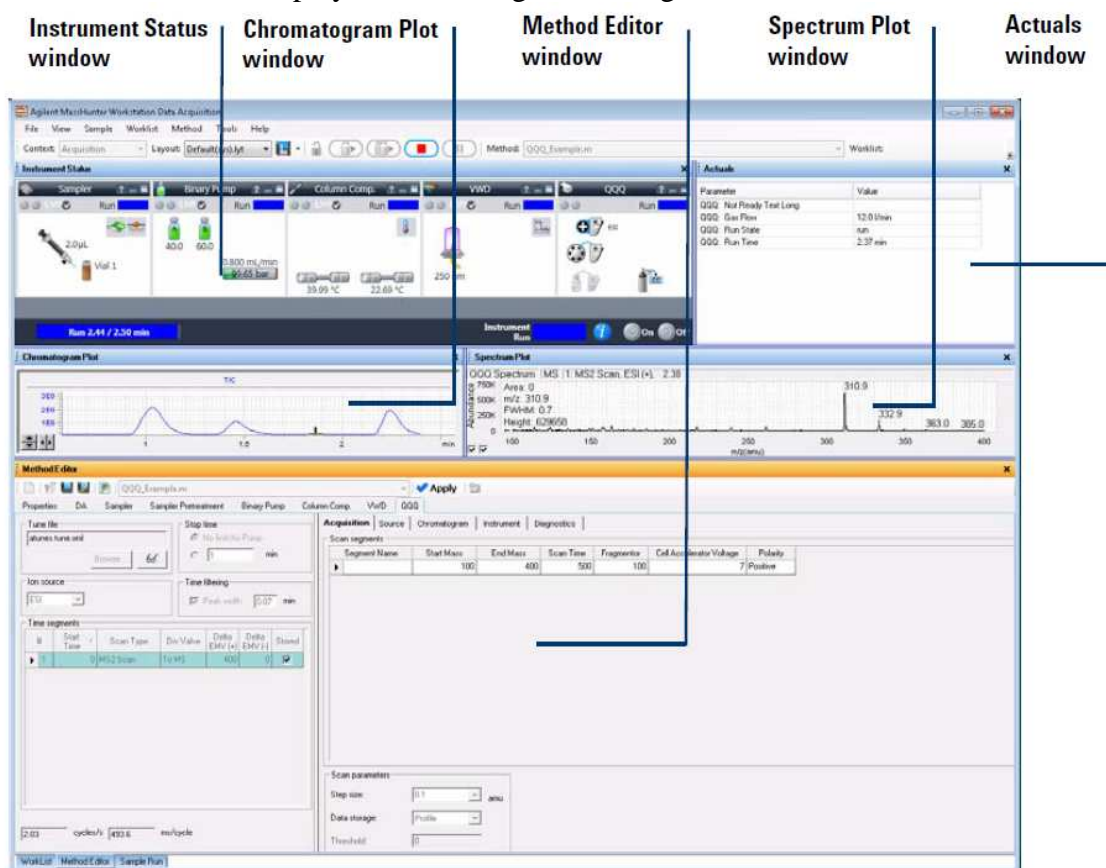
You are not allowed to do the following unless clarified by laboratory manager:

- A. Change solvent reservoirs
- B. Change LC columns
- C. Direct infuse sample into the spray chamber by syringe etc.
- D. Open the electrospray chamber
- E. Use solvent additives not compatible with the MS instrument
- F. Open, run or modify Auto tune or Check tune files

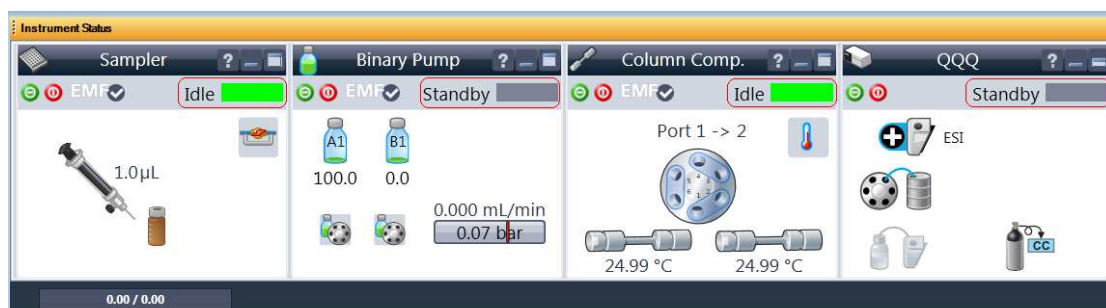
Those that do not follow the rules for operation will immediately be excluded from the using the instrument!

Before you start any experiments on the instrument you should do the following;

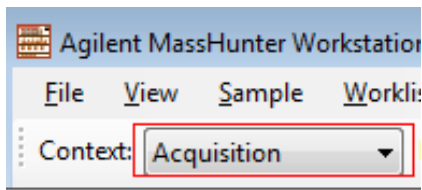
1. Log on to the computer (3000hanover)
2. Confirm that MassHunter Acquisition is running. If not, start the program. Your screen should then display the following controlling units/windows;



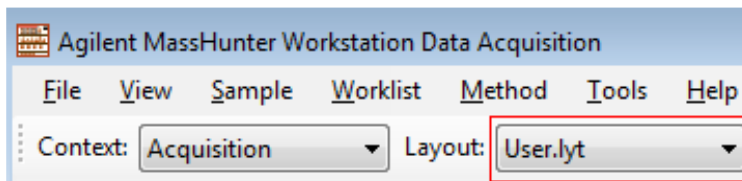
3. Confirm that the sampler and column compartment run in Idle mode and the Binary Pump and QQQ are in Standby mode.



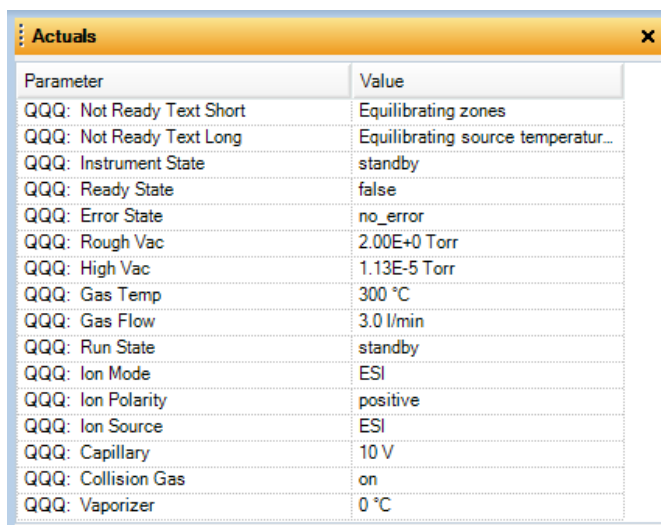
4. Confirm that MassHunter Workstation Data Acquisition runs in acquisition mode.



5. Confirm that the control panel runs with correct layout: User.lyt (Only experienced user should design their layout).



6. Confirm that the MS actual status panel read roughly the following values for **rough vacuum, high vacuum, gas temperature and gas flow** (actual window);



| Parameter | Value |
|---------------------------|------------------------------------|
| QQQ: Not Ready Text Short | Equilibrating zones |
| QQQ: Not Ready Text Long | Equilibrating source temperatur... |
| QQQ: Instrument State | standby |
| QQQ: Ready State | false |
| QQQ: Error State | no_error |
| QQQ: Rough Vac | 2.00E+0 Torr |
| QQQ: High Vac | 1.13E-5 Torr |
| QQQ: Gas Temp | 300 °C |
| QQQ: Gas Flow | 3.0 l/min |
| QQQ: Run State | standby |
| QQQ: Ion Mode | ESI |
| QQQ: Ion Polarity | positive |
| QQQ: Ion Source | ESI |
| QQQ: Capillary | 10 V |
| QQQ: Collision Gas | on |
| QQQ: Vaporizer | 0 °C |

7. Confirm that the LC solvent reservoirs contain enough solvent for your analysis and that solvents are connected to the correct solvent capillary lines.

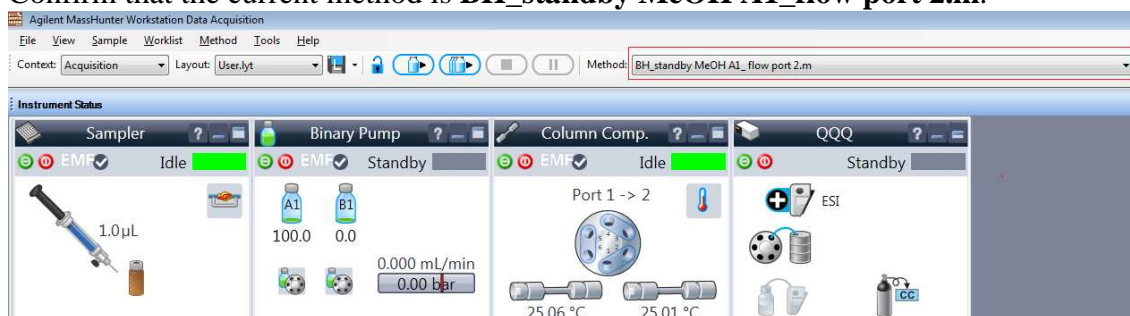
A1: Methanol
A2: H₂O (MilliQ)
B1: Acetonitrile
B2: Optional

8. Check the level of the solvent waste flask and empty this if there is a chance for run over. Use the blue waste can in the hood.

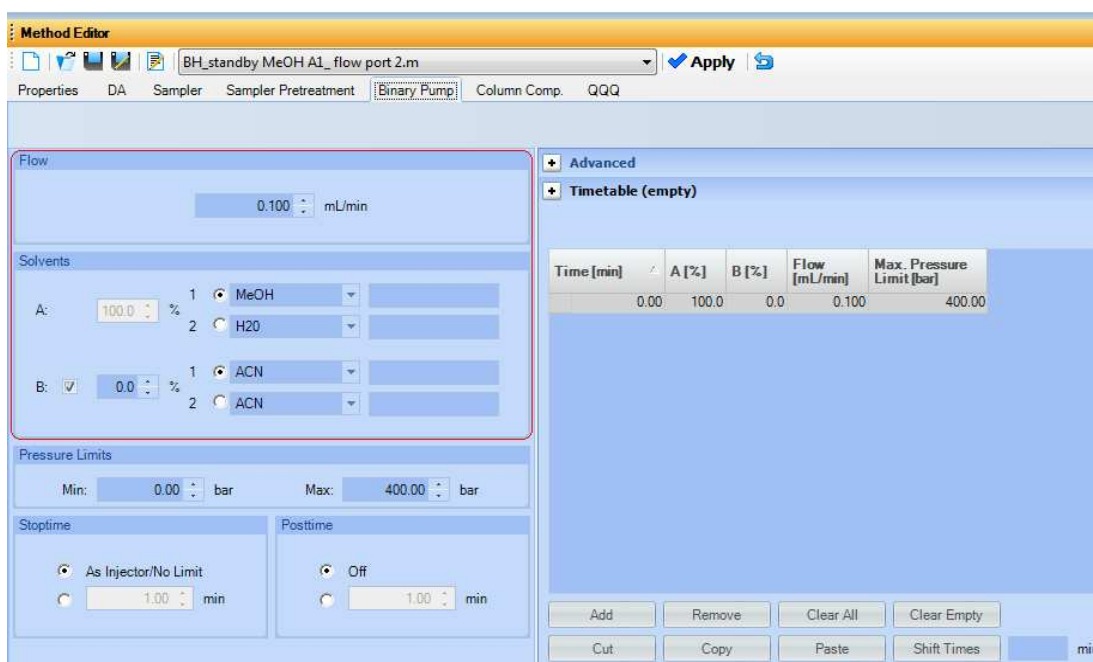
9. Confirm that the LC purge valve is open. If not open it - counter clockwise.

Then;

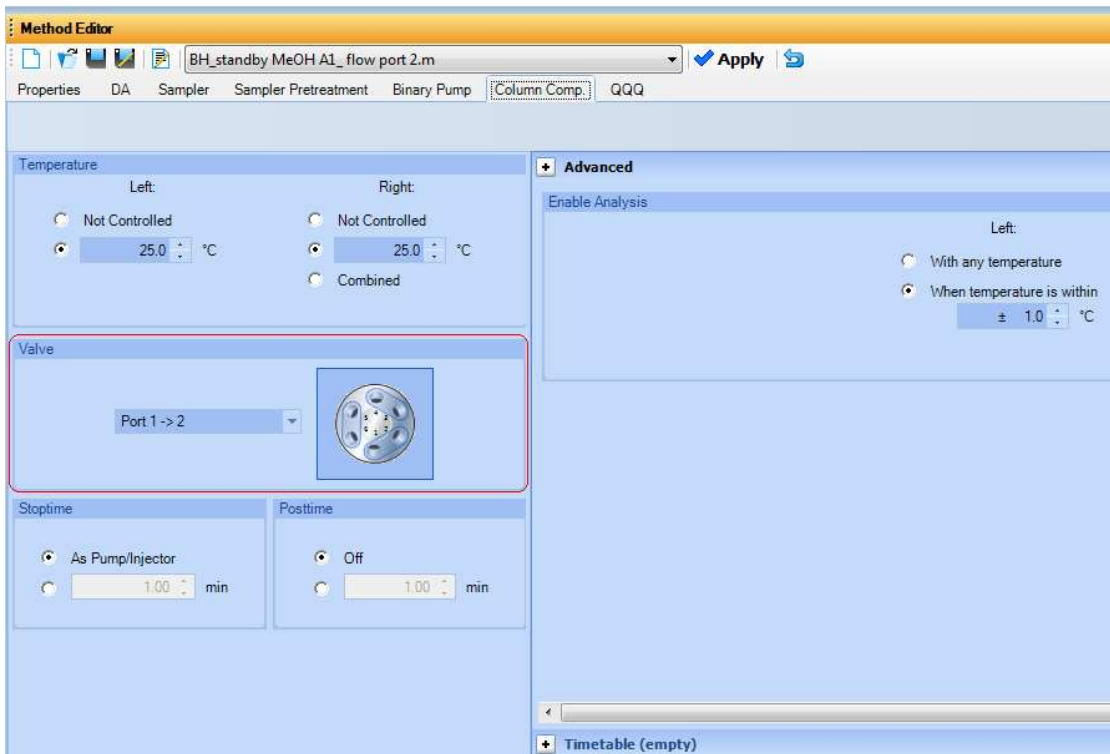
10. Confirm that the current method is **BH_standby MeOH A1_flow port 2.m**.



11. Confirm that the pump delivers solvent from solvent reservoir A1 (methanol) at low solvent flow (0.1 mL/min). You check this under Binary Pump in the Method Editor panel.



12. Confirm that solvent is pumped directly into the solvent flow (infused) through port 1→2. You check this under Column Comp. in the Method Editor.

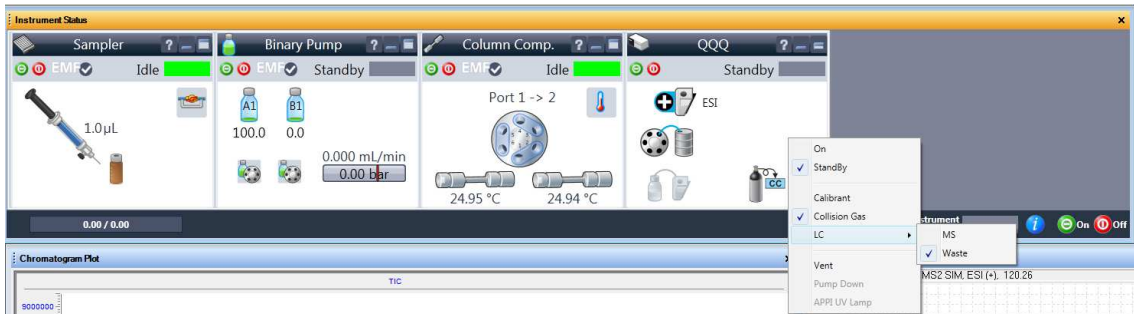


13. Start the pump for purging by clicking the green button in the Binary Pump operating panel.
14. When purging, use a higher flow than the standby flow. Set the solvent flow to 0.6-0.8 mL/min and let the pump run for minimum 5 min using solvent A1 (methanol).

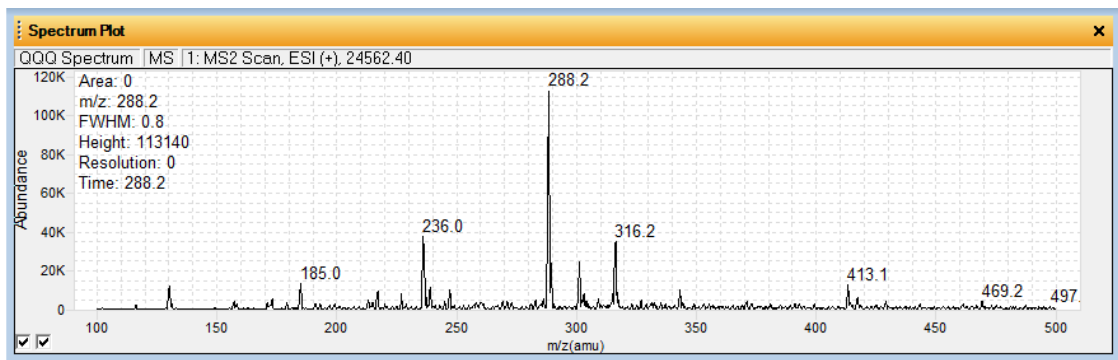
Now it is time to confirm that the MS instrument is operating properly

15. Set the solvent A1 flow to 0.1 mL/min.
16. In the Instrument Status control panel, right mouse click in the QQQ panel and change the instrument status to On.

17. Right mouse click in the QQQ panel and change the diverter flow valve from Waste to MS.



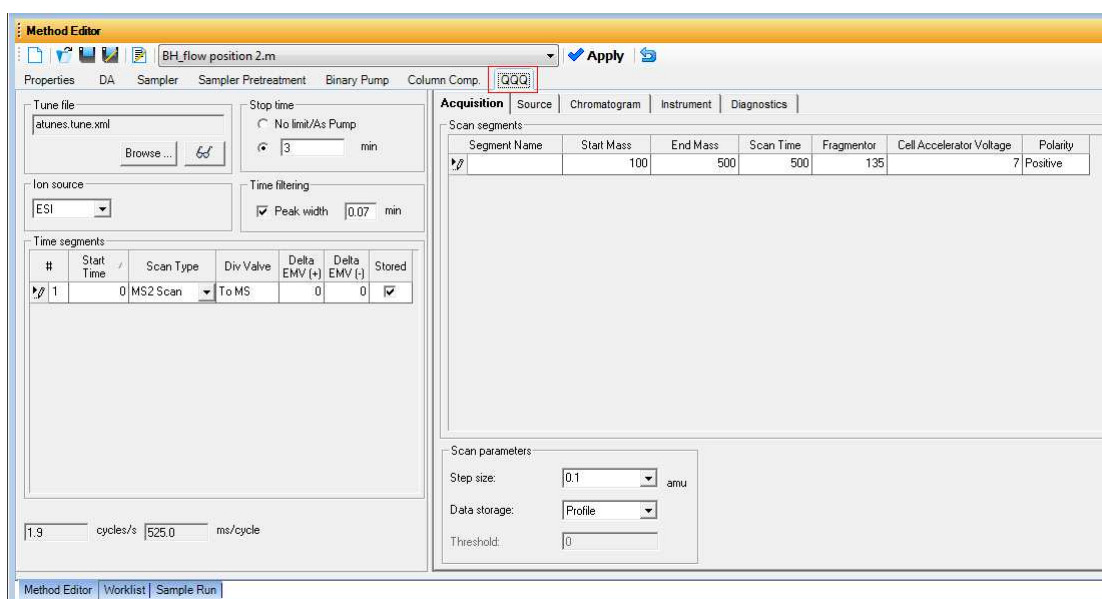
18. Close the LC purge valve (clockwise).
19. Within some minutes you should obtain a real time MS spectrum of your methanol flow including contaminations in the ion source and instrument itself.



Allow the mass spectrum to stabilize. If the stabilized mass spectrum look relative similar to the spectrum above you then right click the QQQ device instrument status window, choose LC > Waste. If it looks dramatically different, the instrument is to contaminated to analyse your sample(s). Contact laboratory manager. If so, right mouse click the QQQ panel and set the instrument status to Standby. Next, stop the LC pump by clicking on the red Stop button on the Binary Pump Instrument Status Operating panel. *If you use any other solvents than methanol the mass spectrum will of course be different.*

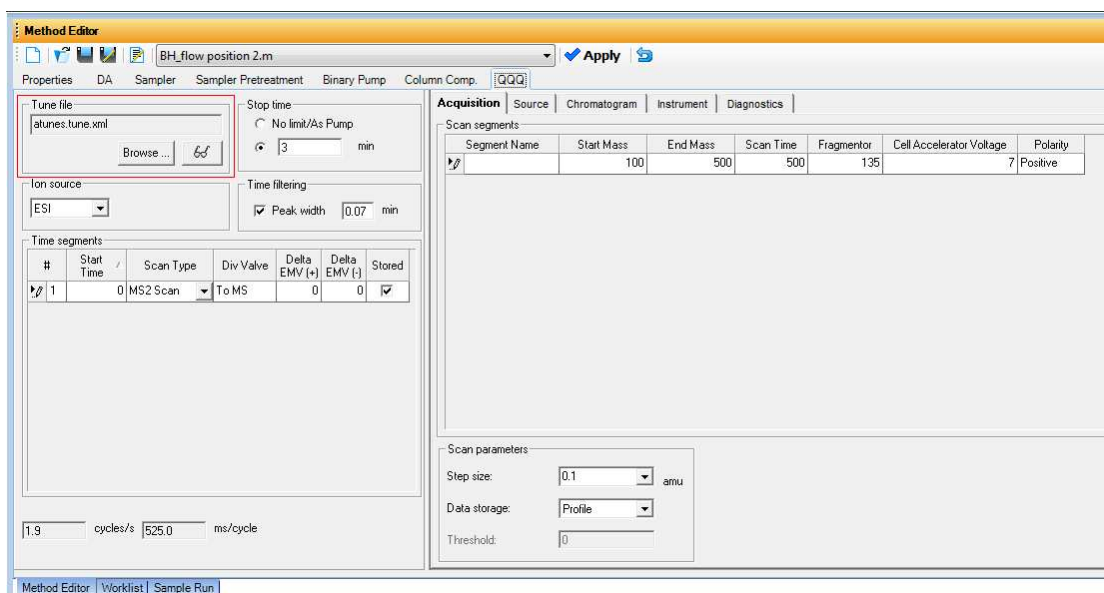
20. Load your method for the experiment you are about to execute. File → load method → your method.

21. Modify your MS method so it accommodates ionisation of your target compound. This is done in the Method editor panel under QQQ.



In the Method Editor panel choose your desired scan type. For acquisition parameters you should strictly limit your scan range for MS2scan experiments and correct value for the fragmentor voltage should be established through test experiments unless you are an experienced user. Values from 60-135 V are typical. For MS2Sim the dwell time must be considered. For MRM experiments the optimal collision energy has to be determined through experiments. For this purpose MassHunter Optimizer software is practical software to start with. The cell acceleration voltage plays an important role for the accumulation time of ions in the collision cell. Especially, for MRM experiments this value must be considered. For MS2Scan and MS2Sim it is safe to use default values. Make sure to have appropriate ion source conditions. Adjust needle voltage (2000-4500 V depending on analyte characteristics, solvent, ion source temperature and nebulizer pressure). *Choice of experimental conditions and combinations of ionisation parameters are closely related to the molecular weight, chemical functionality, polarity and ionisation ability of your target analyte.*

22. Confirm that the tune file read the correct auto tune method. Unless you know what you are doing, you should not change the instrument away from atune.tune.xml.



23. Place your sample vial in a free vial position and make sure that the auto sampler injects from this position.
24. If you are about to run just one single sample, then use the sample run option in the Method Editor panel. For multiple samples, then use the worklist option.
25. When you have finished your analysis, upload the washing method (**BH_washing port 2_B2.m.**) and let it run for 5-10 min. Longer washing times may be required if you have injected many samples. Inspect that ions from your samples disappears through the washing process. The washing method delivers solvent from solvent reservoir B2. This bottle contains a mixture of; methanol(pa) : isopropanol(pa) : water(MilliQ) : acetonitrile(pa) in a 1:1:1:1 ratio. Make sure there is enough of the washing solution for your washing process.
26. When the washing process is finished you should you should inject from a vial containing the same solution as mentioned above. When this injection is done in the washing method it will rinse the column compartment switching valve.
27. Load the standby method (**BH_standby MeOH A1_flow port 2.m.**)
28. Make sure the diverter valve delivers the solvent flow to waste (right mouse click on QQQ and choose LC → waste).
29. Set the triple quadruple (QqQ) to standby (red button).
30. Set the binary pump to standby (red button).

- 31 Check that the capillary is of (a potential of 8-10 V in MS status panel).
- 32 Open the LC purge valve.