Never work on the machine if you have not been trained to operate the machine.
Step 1: Check the machine and login

Before starting your work, please do the following:

a) Check solvents in the reservoir bottles (deionized water and acetonitrile). If the amount of solvents too low, please inform Michal Urban.

b) Login to the operating system. **You will receive the relevant data only after proper training.**

Step 2: Start Data Acquisition software

You see all of the Agilent MassHunter Workstation software icons on your desktop. To start the Data Acquisition program, double-click the **Data Acquisition** icon.

**Note:**

When Data Acquisition opens, the software engines automatically start. If you need to restart them, right-click the Acq System Launcher icon in the system tray and click **Start Engines.**
The windows where you do most of your work

Instrument Status Window  Chromatogram Plot window  Method Editor window  Spectrum Plot window  Actual window

Method Editor, Sample run and Worklist windows are tabbed here. These three windows are “sharing” this space. You click the tab to switch to different window.
Show/hide the windows

You can show one window at a time on the screen or all seven windows. You can never hide all of the windows. To show or hide a window, you click the commands in the View menu. You can also hide a window by clicking the X icon in the upper right corner of the window.

When you click a window, the title of the active window changes to a different colour. Press F1 to obtain help on the active window. You can also drag a window border to resize the window. If you double-click the title of the window, the window “floats” outside of the main window. You can double-click the title bar again to “dock” the window. You can also float and dock the window by right-clicking the title of the window and clicking Floating

Instrument Status window

With this window you view the status of each device configured with the instrument–On, Off or Standby. You also set non-method control and configuration parameters for the LC devices and the MS instrument. You do not need to set up anything, everything is already configured. You can click the ? button in any device panel to get useful informations.

This window displays each device's current status both as text and by its color-coding: (Table 1).
Table 1: Color-coding of Instrument Status Window

<table>
<thead>
<tr>
<th>Color</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Error</td>
</tr>
<tr>
<td>Yellow</td>
<td>Not ready</td>
</tr>
<tr>
<td>Purple</td>
<td>Pre run/Post run</td>
</tr>
<tr>
<td>Blue</td>
<td>Running, Injecting</td>
</tr>
<tr>
<td>Green</td>
<td>Idle</td>
</tr>
<tr>
<td>Dark gray</td>
<td>Offline</td>
</tr>
<tr>
<td>Light gray</td>
<td>Standby (for example, lamps off)</td>
</tr>
</tbody>
</table>

**Actuals window**

With this window you view the current value of selected instrument parameters.
Chromatogram Plot window

With this window you monitor the chromatogram plots in real time. These plots can be user-defined signals and/or instrument parameters.

Spectrum Plot window

With this window you monitor the spectral plots in real time.
Method Editor window

With this window you enter acquisition parameters for the method. There is no need to edit anything; everything is set for the general method!

Sample Run window

With this window you enter sample information to run individual samples interactively, and you can start a single sample run.
**Worklist window**

With this window you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist.

**Tune window**

With this window, you tune the mass spectrometer. You can use one of the automated tuning algorithms, or you can manually tune the instrument.
Step 3: Tune TOF a Q-TOF

a) In the Context list, change the Acquisition mode to Tune mode in the main window at the top left.

The Tune window appears. Only the Instrument Status window, the Actuals window, and the Tune window are available in the Tune context. Note that you tune the TOF separately from the quadrupole.

Tune window
b) Turn on all machine components in the *Instrument Status window*.

![Instrument Status window](image)

Turns on all components.

c) Then click on *Tune and Calibration*

d) Select the polarity of TOF on the *Tune and Calibration* tab. If you are going to tune in Positive polarity, do the following: Click the **Positive** polarity button. If you are going to tune in Negative polarity, do the following: Click the **Negative** polarity button. Next, click *Mass Calibration / Check*.

e) Then click Start TOF Mass Calibration and wait for the report. This may take about 5 minutes. If a report is displayed, the machine calibration has been successful.
f) The calibration is completed; you will switch back to Acquisition mode in the Context list of the main window.
Step 4: Set up and run a method

Before measuring your sample, you must choose an appropriate method of measurement.

a) You select a method in the Method section of the main window.

We have created methods that you can use for routine measurements. Method of first choice is: `general_short_positive(400ul).1ul.m`

This method is suitable for most samples.

Note: If you need to develop a special method for measuring your substance, discuss it first with Michal Urban.

b) Select a method from the tab: `general_short_positive(400ul).1ul.m`

c) Turn on all machine components with the On button. All components of the machine will start work and will be in active mode, which will be shown in green colour in the Instrument Status window.
Step 5: Set up and run worklists

a) Click Worklist

b) Right-click the upper left corner of the worklist to display the following menu.

c) Click Add Multiple Samples.

d) Paste the information into the worklist table, such as sample name, data file name, and path to save the file. The first line of the worklist is always the Blank sample measurement with vial position 1. The measurement of your sample or sample sequence follows. The last step is to wash with the injection of pure acetonitrile in the vial at position 1. Each acquisition (row) is measured for 13 minutes. To add a row to the worklist, right-click in the upper left corner of the worklist to display the menu and select Add Sample.

Poznámka: I recommend measuring Blank sample twice. The first blank measurement removes residue from the previous measurement and the second blank measurement equilibrates the machine.

e) Indicate the position of your sample in the Sample Position (2-99).
f) In the last line, insert a request to stop the pumps after measuring the worklist. It is good to save solvents. Please right-click in the upper left corner of the worklist to display the menu and select Add Script. And select SCP_PumpsAllOff.


The concentration of your sample must be in the range of 10-40 μg/ml. Sample preparation: Dissolve 1 mg of your substance in 1 ml of acetonitrile then take 0.01-0.04 ml of it and dissolve this amount in 1 ml of acetonitrile.
a) Click the **Start Worklist Run** button ( )( ) in the Worklist toolbar.

b) The blank from vial 1 is then injected and measured. Violet colour with Sampler. Then the measurement of your samples continues according to the worklist. Blue colour on all machine components.
c) When all your samples have been measured and the final wash is complete, the pumps will automatically shut down.

**Step 6: Analysis of measured data**

You can analyse your measured samples and look for the appropriate masses in Qualitative Analysis. You can find this program on your desktop

- Use the Qualitative Analysis program to do these steps and more:
  - Review results for acquisition method development
  - Find compounds
  - Identify compounds
  - Do molecular feature extraction
  - Export results