

Agilent 6530 Q-TOF LC/MS User Manual



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**

The screenshot displays the Agilent MassHunter Workstation Data Acquisition software interface. The top menu bar includes File, View, Sample, Worklist, Method, Tools, and Help. Below the menu bar, the 'Context' is set to 'Acquisition' and the 'Layout' is 'Default(sys).lyt'. The 'Method' is 'general_short_posi' and the 'Worklist' is empty.

The **Instrument Status Window** shows the status of various components: Sampler (Idle), Binary Pump (Standby), Column Comp. (Not Ready), VWD (Not Ready), and Q-TOF (Not Ready). The status bar indicates 'Instrument Not Ready'.

The **Chromatogram Plot window** displays a Total Ion Chromatogram (TIC) plot with the y-axis ranging from 0 to 8,000,000 and the x-axis from 0 to 60 minutes. The plot shows a baseline with a small peak around 30 minutes.

The **Method Editor window** shows the 'Method' tab selected. The 'Path' is 'D:\MassHunter\Methods\general_short_positive(400ul).1ul.m' and the 'Est. Run Time' is 17 minutes. The 'Pre Run Script' and 'Post Run Script' fields are empty. The 'Description' field is also empty.

The **Spectrum Plot window** displays a UV-Vis spectrum plot with the y-axis labeled 'mAU' (x10²) ranging from 0 to 1.2 and the x-axis labeled 'nm' ranging from 0 to 105. The plot shows a flat baseline.

The **Actual window** displays a table of parameters and their values:

Parameter	Value
Q-TOF: TOF Vac	1.64E-07 Torr
Q-TOF: Quad Vac	2.43E-05 Torr
Q-TOF: Drying Gas	3.0 l/min
Q-TOF: Error State	-
Q-TOF: Ready State	False
Q-TOF: Rough Vac	2.81E+00 Torr
Q-TOF: Vaporizer/Sheath Gas Temp	125 °C
Q-TOF: Gas Temp	300 °C

At the bottom of the interface, there are three tabs: 'Method Editor', 'Worklist', and 'Sample Run'. The 'Method Editor' tab is currently selected.

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.

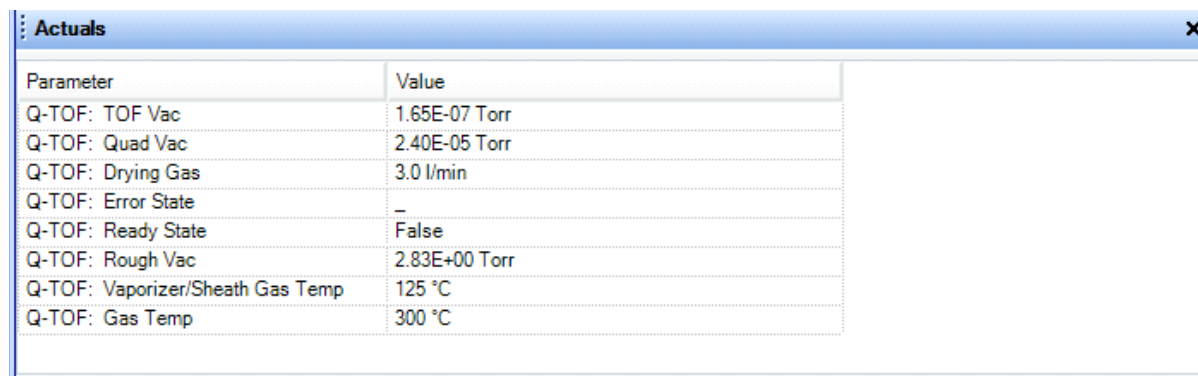
This window displays each device's current status both as text and by its color-coding: (Table 1).

Table 1: Color-coding of *Instrument Status Window*

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

Actuals window

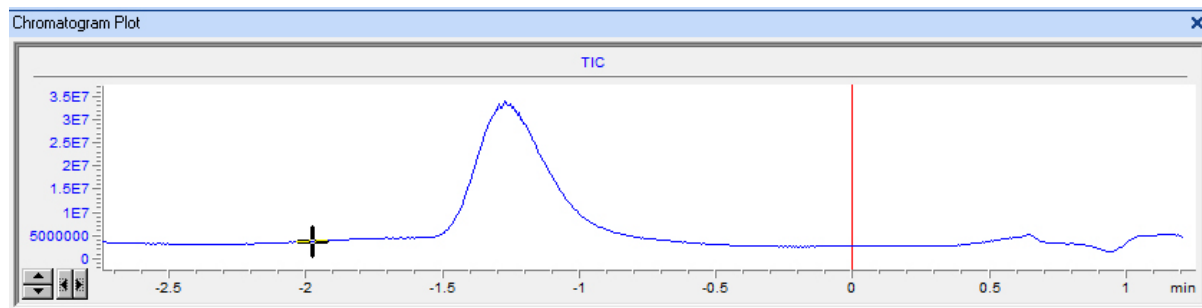
With this window you view the current value of selected instrument parameters.



Actuals	
Parameter	Value
Q-TOF: TOF Vac	1.65E-07 Torr
Q-TOF: Quad Vac	2.40E-05 Torr
Q-TOF: Drying Gas	3.0 l/min
Q-TOF: Error State	—
Q-TOF: Ready State	False
Q-TOF: Rough Vac	2.83E+00 Torr
Q-TOF: Vaporizer/Sheath Gas Temp	125 °C
Q-TOF: Gas Temp	300 °C

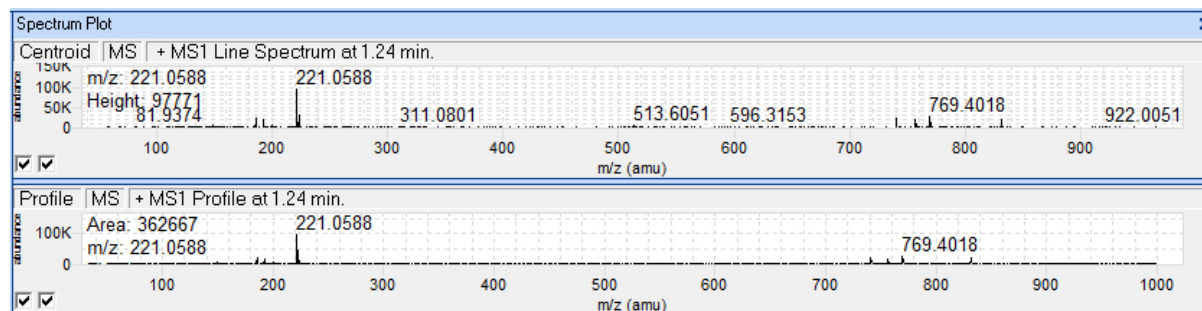
Chromatogram Plot window

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



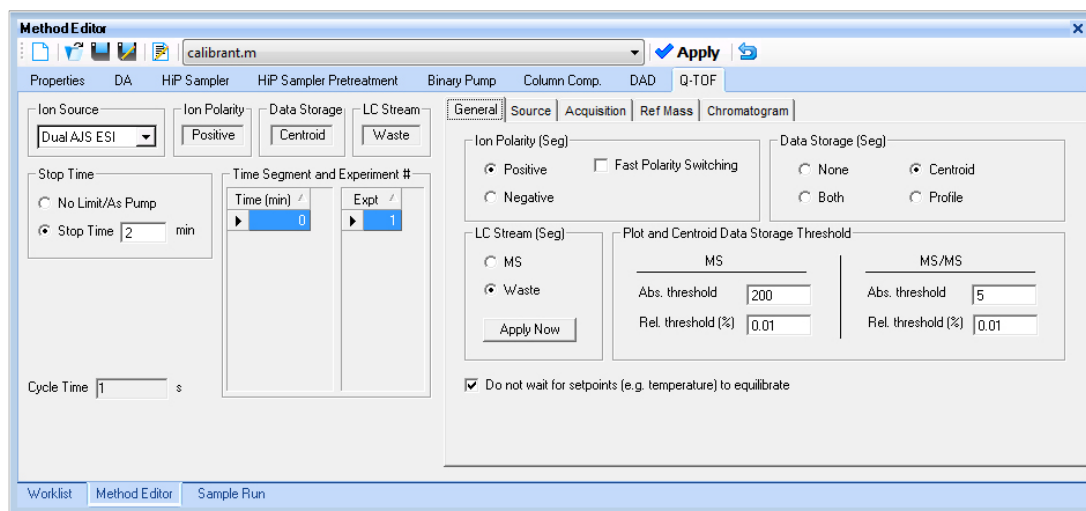
Spectrum Plot window

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



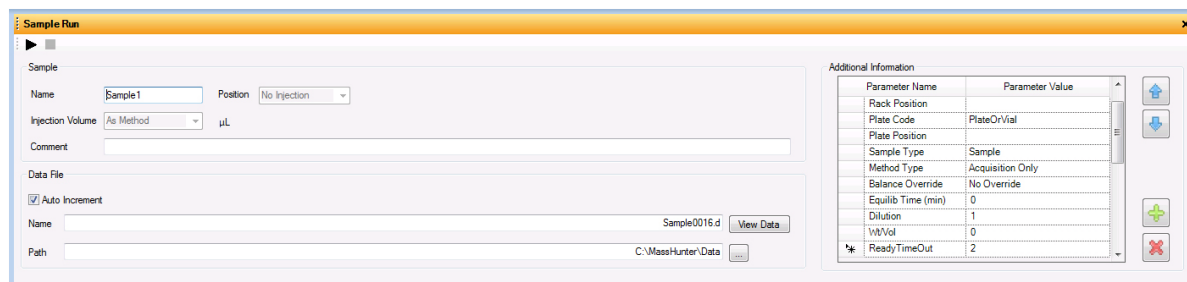
Method Editor window

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.



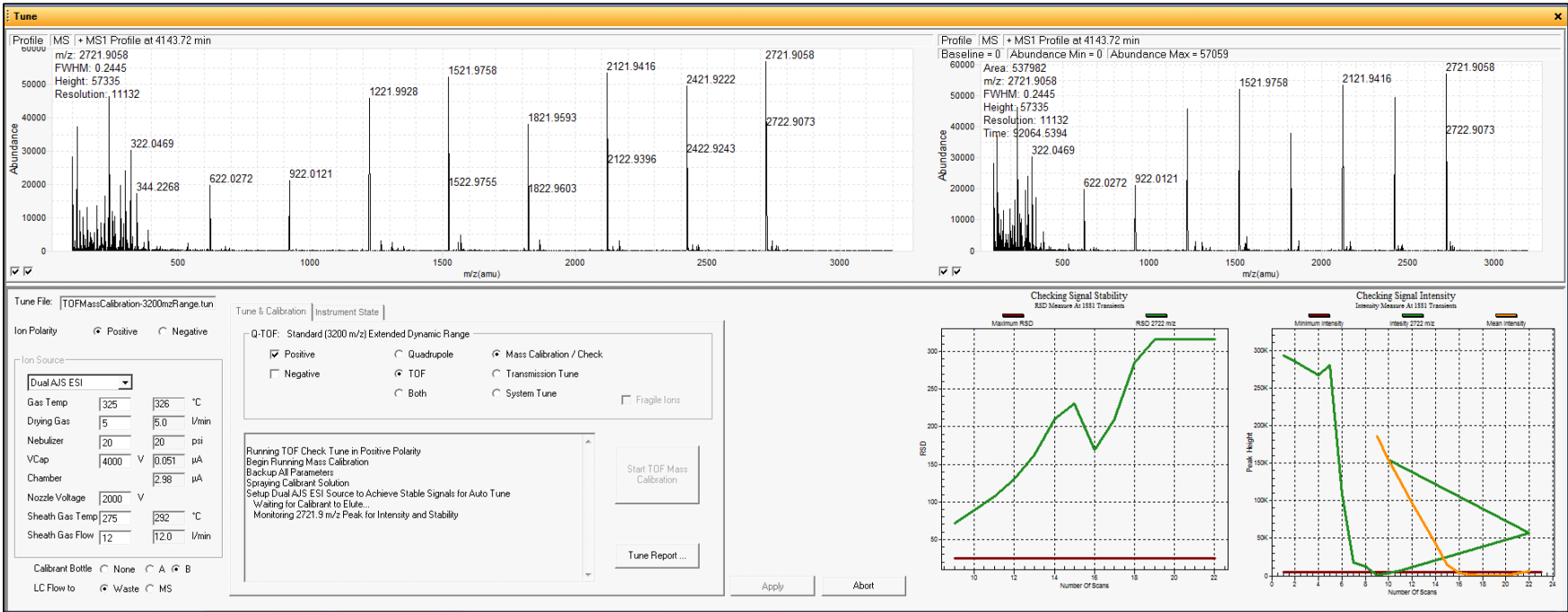
Worklist window

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.

Worklist									
	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment	Sample Group	Info
1	✓ Sulf1	P1-A1	AJSESI_default.m	sulfas001.d	Sample				
2	✓ Sulf1	P1-A2	AJSESI_default.m	sulfas002.d	Sample				
3	✓ Sulf1	P1-A3	AJSESI_default.m	sulfas003.d	Sample				

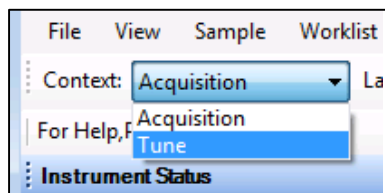
Tune window

With this window, you tune the mass spectrometer. You can use one of the automated tuning algorithms, or you can manually tune the instrument.



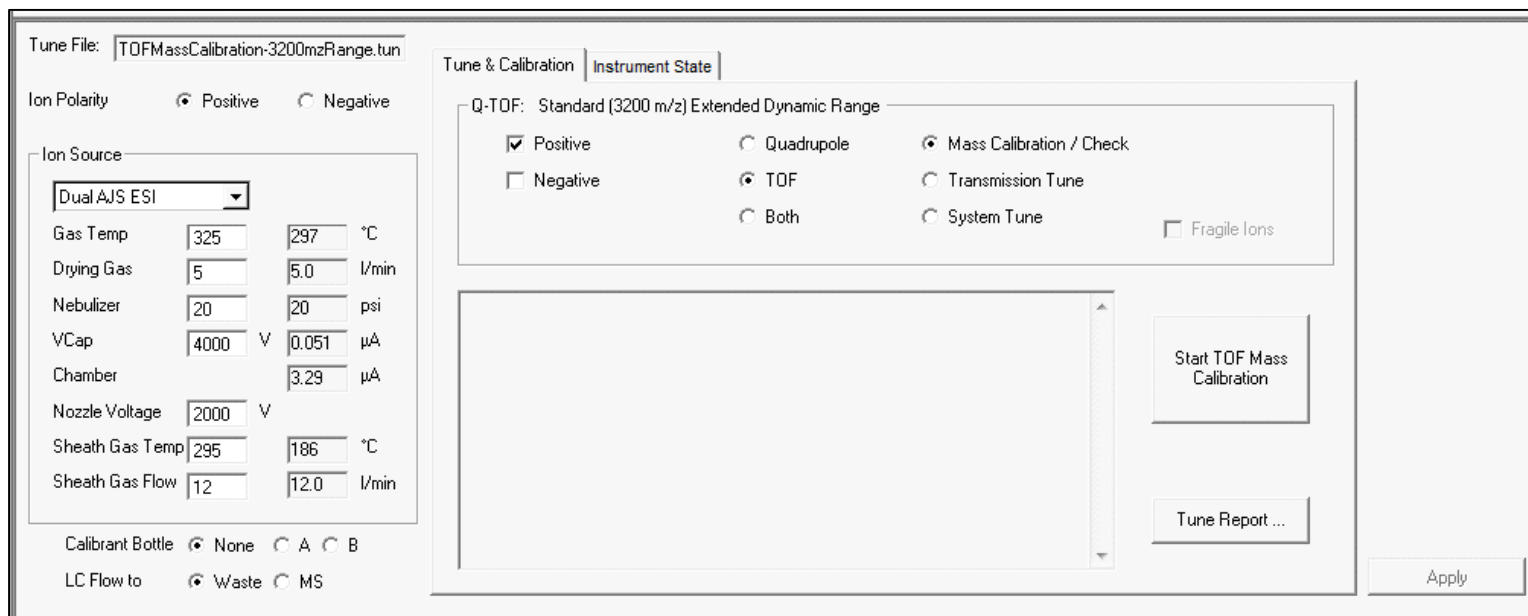
Step 3: Tune TOF a Q-TOF

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

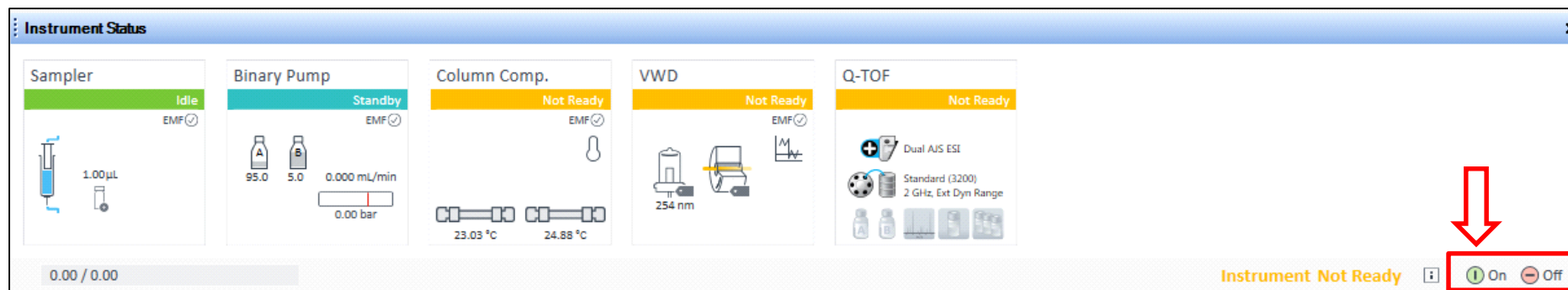


The Tune window appears. Only the *Instrument Status* window, the *Actuals* window, and the *Tune* window are available in the *Tune* context. Note that you tune the TOF separately from the quadrupole.

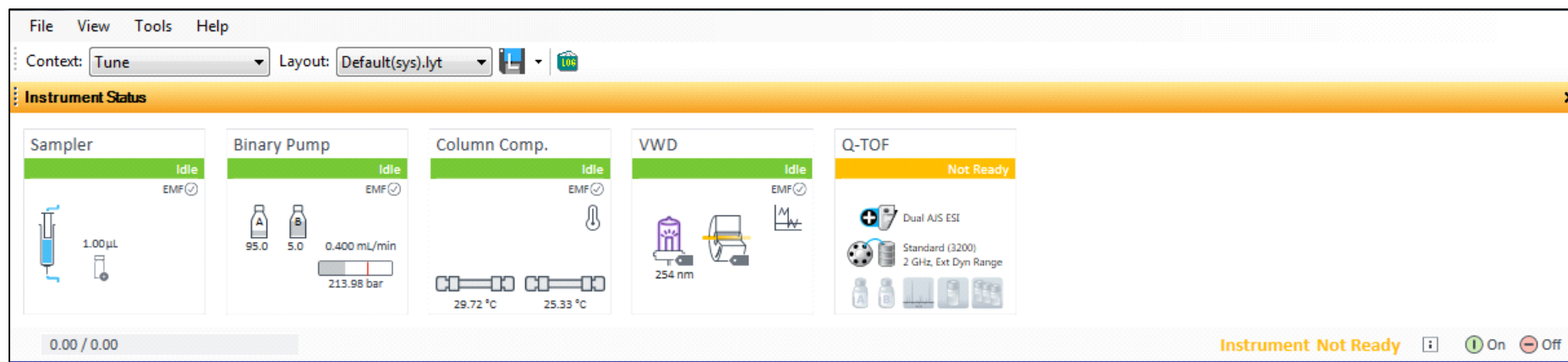
Tune window

A screenshot of the 'Tune' window in the software. The window has a title bar and a menu bar with 'Tune & Calibration' and 'Instrument State'. The 'Tune & Calibration' menu is active. On the left, there's a 'Tune File:' field with 'TOFMassCalibration-3200mzRange.tun'. Below it are 'Ion Polarity' options: 'Positive' (selected) and 'Negative'. Under 'Ion Source', a dropdown menu shows 'Dual AJS ESI'. Below this are various parameters with input fields: 'Gas Temp' (325, 297 °C), 'Drying Gas' (5, 5.0 l/min), 'Nebulizer' (20, 20 psi), 'VCap' (4000 V, 0.051 µA), 'Chamber' (3.29 µA), 'Nozzle Voltage' (2000 V), 'Sheath Gas Temp' (295, 186 °C), and 'Sheath Gas Flow' (12, 12.0 l/min). At the bottom left are 'Calibrant Bottle' options (None, A, B) and 'LC Flow to' options (Waste, MS). The main area on the right is titled 'Q-TOF: Standard (3200 m/z) Extended Dynamic Range'. It contains radio buttons for 'Positive' (checked), 'Negative', 'Quadrupole', 'TOF' (checked), 'Both', 'Mass Calibration / Check' (checked), 'Transmission Tune', and 'System Tune'. There's also a 'Fragile Ions' checkbox. Below this is a large empty rectangular area. To the right of this area are two buttons: 'Start TOF Mass Calibration' and 'Tune Report ...'. At the bottom right is an 'Apply' button.

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



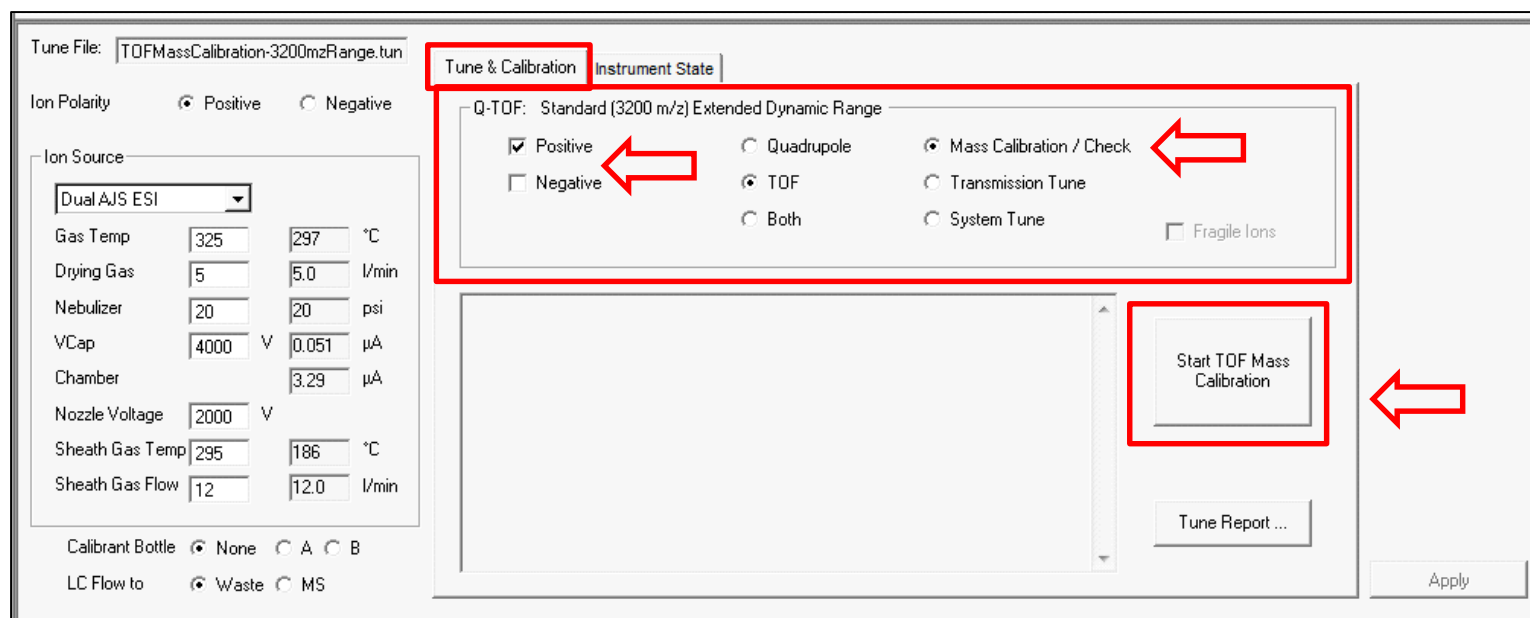
Turns on all components.



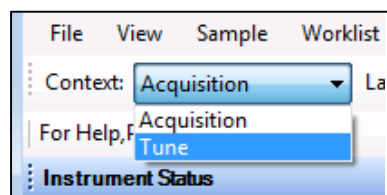
c) Then click on *Tune and Calibration*

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.



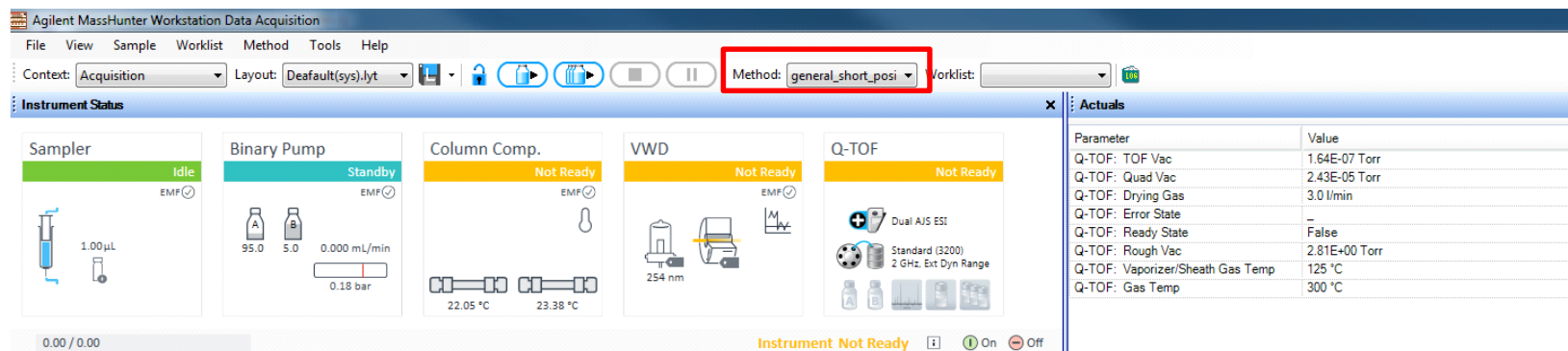
f) The calibration is completed; you will switch back to *Acquisition* mode in the *Context list* of the main window.



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.

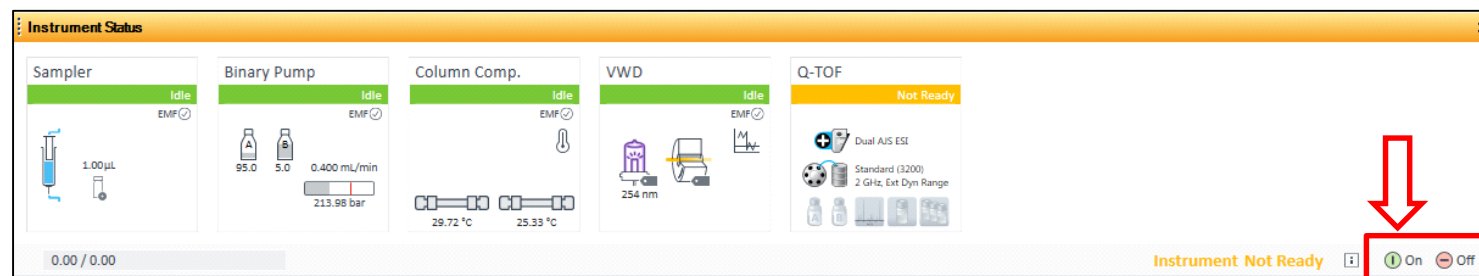


We have created methods that you can use for routine measurements. Method of first choice is: *general_short_positive(400ul).1ul.m*

This method is suitable for most samples.

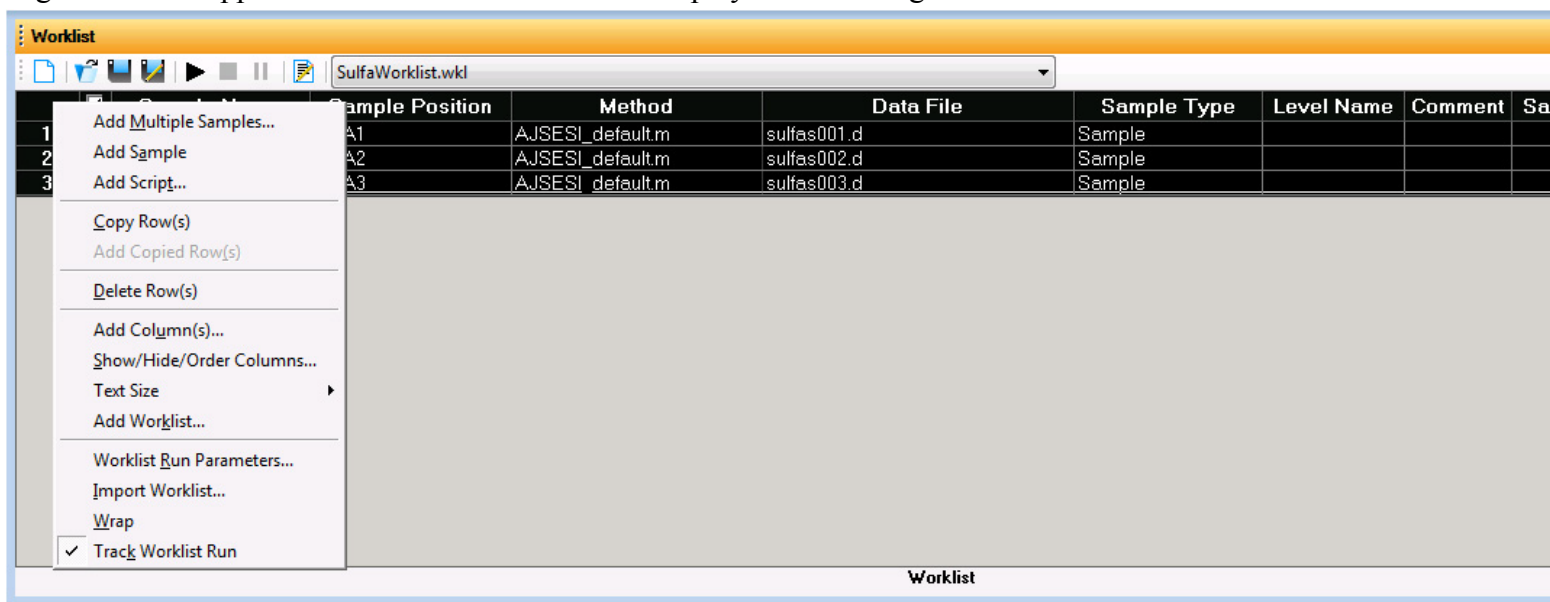
Note: If you need to develop a special method for measuring your substance, discuss it first with Michal Urban.

- 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

- Click Worklist
- Right-click the upper left corner of the worklist to display the following menu.



- Click *Add Multiple Samples*.
- 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.

- Indicate the position of your sample in the *Sample Position* (2-99).

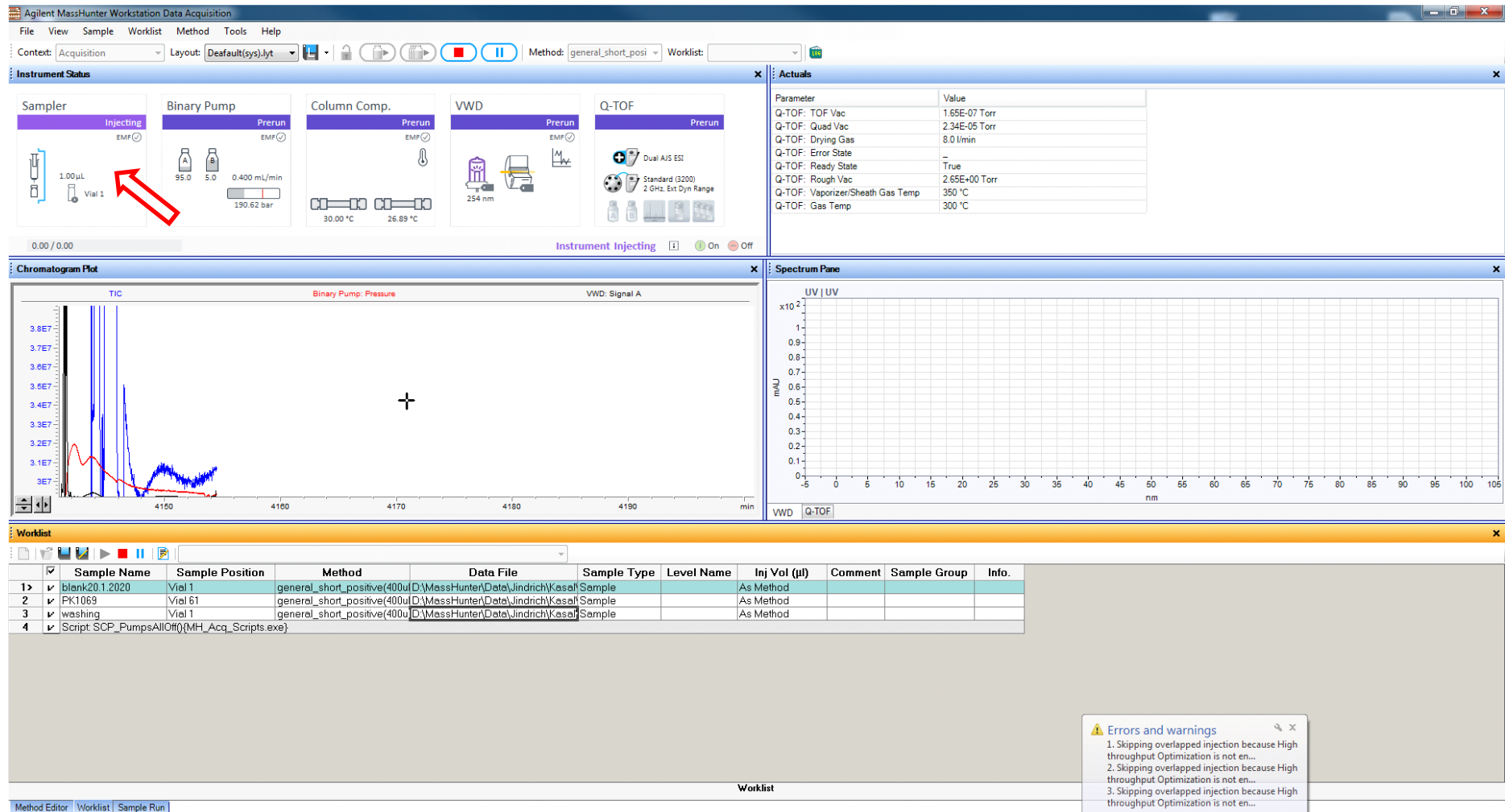
Worklist											
	✓	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (µl)	Comment	Sample Group	Info.
1>	✓	blank20.1.2020	Vial 1	general_short_positive(400u	D:\MassHunter\Data\Jindrich\Kasa\	Sample		As Method			
2	✓	PK1069	Vial 61	general_short_positive(400u	D:\MassHunter\Data\Jindrich\Kasa\	Sample		As Method			
3	✓	washing	Vial 1	general_short_positive(400u	D:\MassHunter\Data\Jindrich\Kasa\	Sample		As Method			
4	✓	Script: SCP_PumpsAllOff()\{MH_Acq_Scripts.exe}									

- 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*.
- g) The sample must be completely dissolved in Agilent vials (<https://www.agilent.com/store/productDetail.jsp?catalogId=5190-4034>) with a cap having a septum (<https://www.agilent.com/store/productDetail.jsp?catalogId=5182-0717>). **Never use different septum !!!**



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.



Agilent MassHunter Workstation Data Acquisition

File View Sample Worklist Method Tools Help

Context: Acquisition Layout: Default(sys).jyt Method: general_short_posi Worklist:

Instrument Status

Sampler Injecting EMP 1.00 µL Vial 1

Binary Pump Prerun EMP 95.0 5.0 0.400 mL/min 190.62 bar

Column Comp. Prerun EMP 30.00 °C 26.89 °C

VWD Prerun EMP 254 nm

Q-TOF Prerun EMP Dual AJS ESI Standard (3200) 2 GHz Ext Dyn Range

Actuals

Parameter	Value
Q-TOF: TOF Vac	1.65E-07 Torr
Q-TOF: Quad Vac	2.34E-05 Torr
Q-TOF: Drying Gas	8.0 l/min
Q-TOF: Error State	-
Q-TOF: Ready State	True
Q-TOF: Rough Vac	2.65E+00 Torr
Q-TOF: Vaporizer/Sheath Gas Temp	350 °C
Q-TOF: Gas Temp	300 °C

Chromatogram Plot

TIC Binary Pump: Pressure VWD: Signal A

Spectrum Pane

UV | UV x10² mAU nm

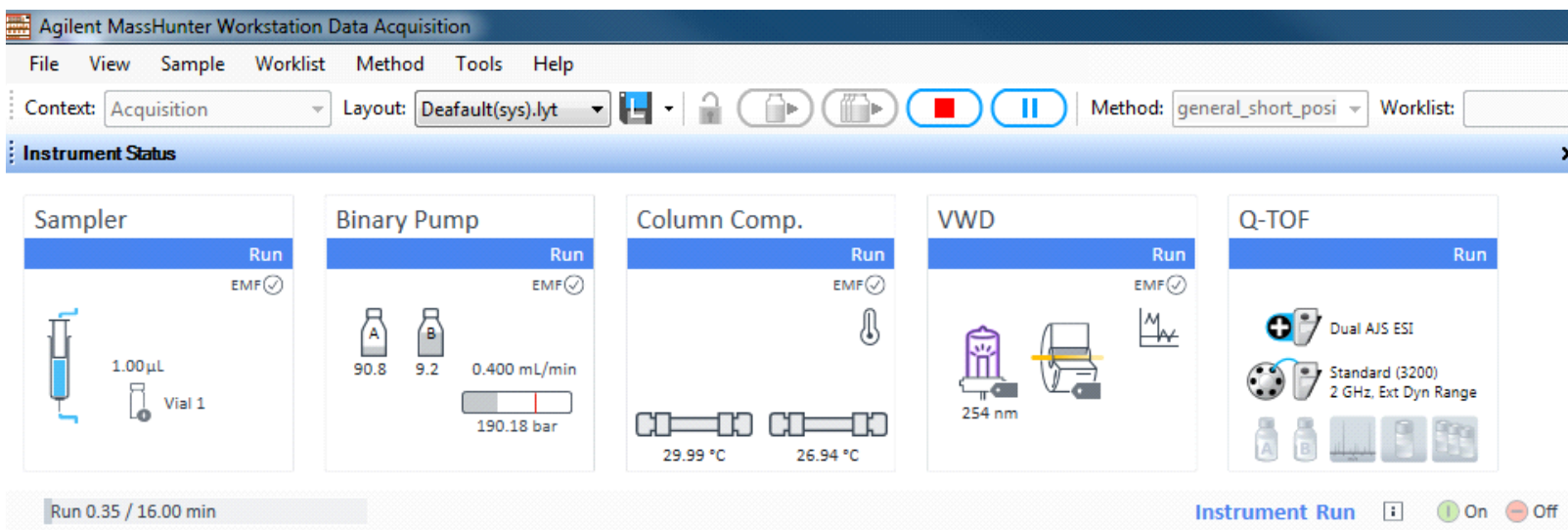
Worklist

	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (µl)	Comment	Sample Group	Info.
1	blank20.1.2020	Vial 1	general_short_positive(400ul)	D:\MassHunter\Data\Jindrich\Kasa\Sample	As Method					
2	PK1069	Vial 61	general_short_positive(400ul)	D:\MassHunter\Data\Jindrich\Kasa\Sample	As Method					
3	washing	Vial 1	general_short_positive(400ul)	D:\MassHunter\Data\Jindrich\Kasa\Sample	As Method					
4	Script: SCP_PumpsAllOff({MH_Acq_Scripts.exe})									

Errors and warnings

1. Skipping overlapped injection because High throughput Optimization is not en...
2. Skipping overlapped injection because High throughput Optimization is not en...
3. Skipping overlapped injection because High throughput Optimization is not en...

Method Editor Worklist Sample Run



- 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