



Department of Chemistry  
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## **LC-MS User Guide**

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Agilent 1260 Infinity II Liquid Chromatograph  
6520B QTOF Mass Spectrometer



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## General Information

1. Only trained users may operate this instrument. New users should contact Dr. Blauch ([dablauch@davidson.edu](mailto:dablauch@ davidson.edu)) to arrange for training. **All users must be trained by Dr. Blauch.**
2. The LC-MS was purchased with a grant from the National Science Foundation MRI program. All publications reporting data obtained with this instrument should include an acknowledgement of the NSF MRI grant (CHE-1624377).
3. Instrument availability is managed with a Google Calendar (Davidson College LC-MS Schedule):  
<https://calendar.google.com/>  
A Google account is required to access this calendar. (All users with a Davidson College e-mail address have a Google account.) All trained users have permission to make entries on this calendar.

Unless otherwise reserved, the instrument is available for walk-up use.

Before walk-up use of the instrument, consult the on-line calendar for instrument availability.

4. The mass spectrometer is normally equipped with an ESI ion source. If you require the APCI ion source, contact Dr. Blauch well in advance of your scheduled time.
5. Weekly maintenance and system checks are performed by Dr. Blauch (see the on-line calendar). Promptly report any problems with the instrument to Dr. Blauch.
6. LC-MS data files are very large (hundreds of MB or larger). For each experiment, a series of files are stored in a folder under D:\MassHunter\Data. (Data folders have the .d extension.) To avoid using all available disk space and creating excessively large backup sets, **please delete all data files that are no longer needed at the end of each session.**

## Instrumentation

### Agilent 1260 Infinity II Liquid Chromatograph

For LC-MS applications, the following chromatographic conditions are typically used:

Injection Volume: 0.1 – 5.0  $\mu$ L

Flow Rate: 0.1 – 0.5 mL/min

ESI and APCI ion sources must quickly and completely vaporize the mobile phase coming off the column. A flow rate that is too high results in incomplete vaporization which leads to arcing of the electrodes, producing spectral artifacts and potentially damaging the hardware.

House Column: Zorbox Extend-C-18 2.1x50 mm, 1.8  $\mu$ m  
S/N USHBD05996 maximum pressure 600 bar  
Agilent Part Number 727700-902

A restriction capillary is also available for flow-injection analysis mass spectrometry.

The system has four solvent reservoirs.

- |   |                          |  |
|---|--------------------------|--|
| A | User Solvent (Aqueous)   | (The brown bottles are for aqueous solutions.) |
| B | User Solvent (Organic)   |  |
| C | 80% methanol / 20% water | (System is washed with the solution.)          |
| D | User Solvent             | (Often used for LC cleaning.)                  |

See **Solvents and Electrolytes** (below) for a list of mobile phases that are compatible with ESI and APCI ion sources.

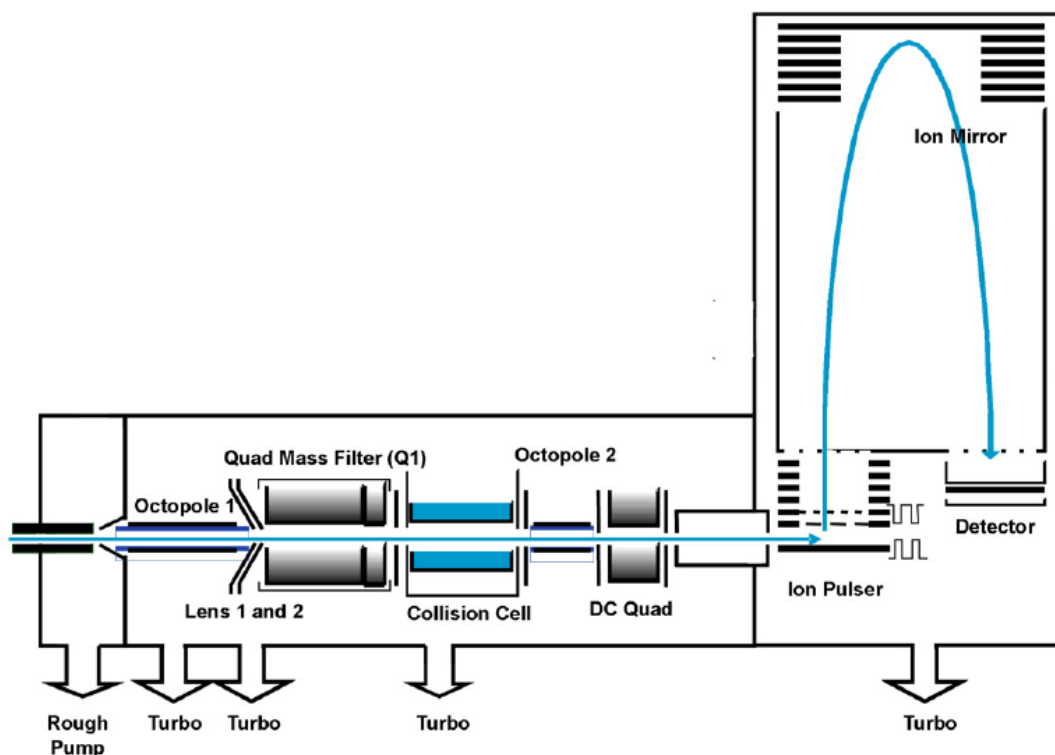
The solution of 80% methanol in water (Reservoir C) is used to wash the LC and the ion source before the instrument is placed in standby mode. Do not alter the composition of Reservoir C.

The pump is equipped with a seal wash option, which must be activated whenever the pump is in use. The seal wash should always use 10% isopropanol in water, which is stored in a separate bottle on top of the LC.

The autosampler includes a needle wash option, which is used to remove traces of sample solutions from the outside of the needle between injections. The needle wash solution should be a good solvent for the sample materials. The 80% methanol solution (Reservoir C) is usually used for this purpose.

The temperature can be controlled in both the auto-sampler compartment and the column compartment.

## Agilent 6520B QTOF Mass Spectrometer



*QTOF Concepts Guide, Agilent Technologies, 2014.*

The 6520 QTOF mass spectrometer consists of three main units: a quadrupole mass filter (Q), a collision cell, and a time-of-flight (TOF) mass analyzer.

The [quadrupole](#) (Q1) can be used to select a narrow  $m/z$  range of ions for subsequent analysis. For simple mass spectra (MS rather than MS/MS), the quadrupole is set to pass all ions.

The [collision cell](#) can be used to fragment ions by accelerating the ions into collisions with  $N_2$  gas. Although ESI is a soft ionization technique (see below), one can use the collision cell to cause fragmentation when desired (MS/MS experiments).

The [time-of-flight mass spectrometer](#) (far right in the figure above) measures the mass-to-charge ratio by measuring the time it takes for an ion to travel across the flight tube (the tall tower on the right of the instrument). Under ideal conditions, this reflecting TOF technology provides  $m/z$  values to a precision of 2 ppm in the range 50 – 3200. The maximum resolving power is 20,000, but in practice, 10,000 to 16,000 is typical when the instrument is in the extended dynamic range. The resolution is approximately 0.05 for  $m/z$  near 500.

### Modes

1. The QTOF normally operates in standard "3200" mode (m/z 50 - 3200). There is also a low mass "1700" mode and a high mass "20000" mode.
2. The instrument is in extended dynamic range (2 GHz) by default. A high resolution (4 GHz) and an extended mass (1 GHz) range are also available.
3. The mass spectrometer can operate in either positive ion or negative ion mode.  
(Do not use the fast polarity switching mode.)

Consult Dr. Blauch if you wish to use a setting other than [3200/extended dynamic range](#).

It takes 0.100 msec for the QTOF to acquire a full mass spectrum. If the system is set to acquire 1 spectrum/sec, then each spectrum is sum of nearly 10,000 individual measurements. The extensive signal averaging greatly improves the precision.

### Detector Saturation

The maximum signal at any m/z value in each individual 0.100 msec acquisition is 255 in standard (4 GHz) range or  $255 (12) = 3060$  in extended dynamic (2 GHz) range.

Suppose 1 spectra/sec is acquired (this value is set in the acquisition method). Each displayed spectrum will contain approximately 10000 individual acquisitions acquired over a 1 sec period.

The maximum signal for any peak in the spectrum is called the **hard saturation limit**. For signals that are inherently more intense this limit, the signal is simply truncated. For the above acquisition rate, the hard saturation limit is

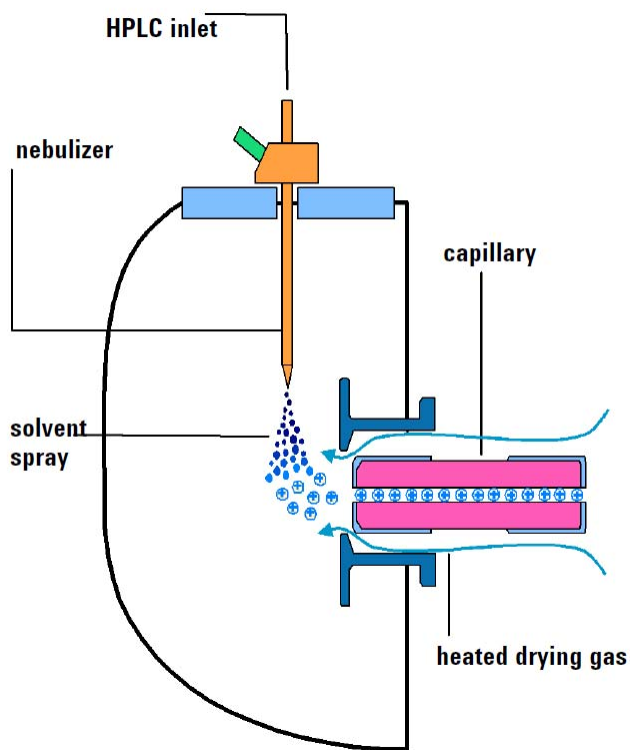
4 GHz Mode:	$10000 (255)$	$= 2550000$	$= 2.6 \text{ M}$
2 GHz Mode:	$10000 (3060)$	$= 30600000$	$= 31 \text{ M}$

In practice, the detector response is only linear up to about 20% of the hard saturation limit.

This lower limit is called the **soft saturation limit**. In the above example, the soft saturation limits are 500 K (4 GHz mode) or 6 M (2 GHz mode). If a peak in a spectrum exceeds the soft saturation limit, it is flagged with a \* in the Qual program.

For samples with strong signals, check the peak intensities to be sure the soft saturation limit is not exceeded. A signal beyond the soft saturation limit will not give a linear calibration curve and the m/z at the top of the peak may be in error owing to distortion of the peak shape.

## ESI Ion Source



QTOF Concepts Guide, Agilent Technologies, 2014.

Electrospray ionization (ESI) creates ions by dispersing the mobile phase as a fine mist (nebulizer). Hot nitrogen gas is blown through the aerosol to evaporate the solvent.

The tip of the nebulizer is an electrode at ground. While the end of the capillary is between 2000 and 5000 V (this potential is called the capillary voltage,  $V_{cap}$ ). The spray shield surrounding the nebulizer is poised at 500 V. The large potential difference between the nebulizer tip and the capillary produces charged droplets in the aerosol ("solvent spray"), that is, the droplets have excess positive or negative charge (depending upon the sign of  $V_{cap}$ , which depends upon whether the instrument is operating in positive or negative mode).

As solvent evaporates from the droplets, the charge density in the droplets increases. When the charge density reaches  $\approx 10^8 \text{ V/cm}^3$ , individual ions are ejected from the droplets.

Ion creation can occur in solution or in the gas phase. The chemistry of the mobile phase plays an important role in ion formation. Most neutral compounds form ions by being protonated (ESI+) or deprotonated (ESI-), though adducts with sodium, potassium, ammonium, formate, or acetate ion are also possible. ESI works best for ionic and polar compounds, especially those that are readily protonated or deprotonated. Nonpolar compounds often give little or no signal.

If a compound has multiple, widely separated acid-base sites, a multiply charged ion can form. Because the mass spectrometer measures the mass-to-charge ratio ( $m/z$ ) rather than the mass itself ( $m$ ), multiply charged ions appear in the mass spectrum at an integer fraction of the mass. One can identify the charge on an ion by examining the spacing between isotope peaks. The carbon-13 isotope peak appear 1 amu higher in mass than the mono-isotopic peak. Thus the spacing between isotope peaks is 1.00 for a +1 ion, 0.500 for a +2 ion, 0.333 for a +3 ion, etc.

Electrospray ionization (ESI) is a soft ionization method, which means that fragmentation of the molecular ion is generally not observed. Thus one expects a single peak (with associated isotope peaks) for a given compound.

Positive Ion Mode: Solvent pH usually 2 – 7

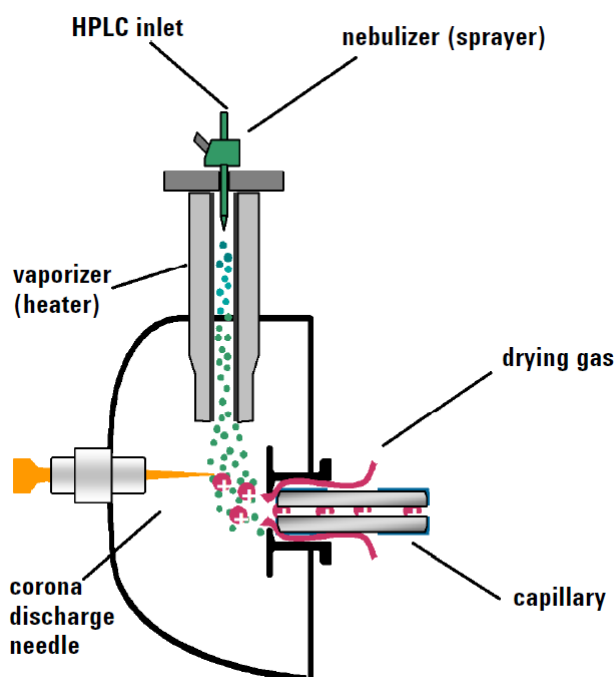
For neutral compounds with basic sites (the most common scenario), it is helpful to include 0.1% formic acid in the mobile phase to aid protonation of the analyte.

Negative Ion Mode: Solvent pH usually 7 – 10

For neutral compounds with acidic sites, it is helpful to include 0.1% ammonium acetate in the mobile phase, though quality ESI- spectra can sometimes be obtained with 0.1% formic acid.

Because the solvent must be rapidly evaporated to produce gas-phase ions, the mobile phase (and any electrolytes in the mobile phase) must be volatile.

## APCI Ion Source



QTOF Concepts Guide, Agilent Technologies, 2014.

In atmospheric pressure chemical ionization (APCI), the mobile phase is also nebulized. The aerosol droplets are heated to evaporate the solvent. The gas-phase mixture of solvent and analytes is passed through an electrical discharge (corona discharge) which ionizes some of the solvent molecules. The ionized solvent molecules behave as reagent ions and protonate (or deprotonate) the analyte.

Because ionization occurs in the gas-phase, the analyte must be volatile. Thus APCI is well-suited for low-molecular weight polar and non-polar compounds but not ionic compounds. Chemical ionization produces a singly charged ion.

As with ESI, only volatile solvents and electrolytes may be used. Also like ESI, the mobile phase chemistry is important for effective ionization of analytes. For APCI, the solvent should be a good charge-transfer reagent.

Methanol is a good choice. Do not use acetonitrile, which works poorly and leaves carbon residue in the ion source. Chlorinated solvents can enhance ionization in negative-ion mode (APCI-), but should only be used in low fractions.

APCI is a semi-soft ionization technique. The molecular ion often dominates the mass spectrum, but fragmentation can occur.



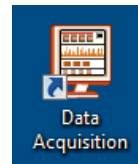
## MassHunter

MassHunter is the Agilent software used to control the LC-MS, execute experiments, collect data and perform data analysis. This software is installed on the LC-MS computer and on the computer in the back left of the instrumentation lab (Tag 15296, Qual and Quant but not Acq).

MassHunter consists of three programs: **Acq**, **Qual**, and **Quant**. Each program has its own method.

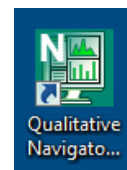
### Data Acquisition (B.08.00) (red icon)

The acquisition module (Acq) permits tuning and calibration of the QTOF, configuration of the LC and QTOF, and set up and execution of experiments. Each experiment is controlled by a method, which configures all elements of the hardware.



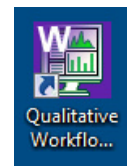
### Qualitative Analysis: Navigator (B.08.00) (green icon)

The qualitative navigator module (Qual Navigator) permits examination of chromatograms, extraction of spectra, baseline corrections, and peak integration. The focus of this module is manipulation of chromatograms.



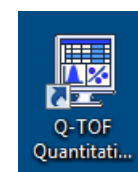
### Qualitative Analysis: Workflow (B.08.00) (purple icon)

The qualitative workflow module (Qual Workflow) is designed to search for compounds and identify their chemical composition based upon the mass spectrometry data.



### Quantitative Analysis (B.08.00) (blue icon)

The quantitative module (Quant) is used to prepare calibration curves and determine analyte concentrations based upon a set of data for a series of standard and sample solutions. This software is especially well-suited for quantification of large amounts of data.



Each user's research group should have its own folder under **D:\MassHunter\Data** and **D:\MassHunter\Methods** (for example, D:\MassHunter\Data\Blauch).

A shortcut to the **D:\MassHunter\Data** folder (Data) is on the desktop.

**D:\MassHunter** is shared on the network as <\\QTOF-PC\MassHunter>.

The D:\MassHunter folder is backed up daily.

In July 2017, the version of MassHunter was updated.

Data and methods from the previous version of MassHunter are stored under D:\MassHunter-old 6.0

## Solvents and Electrolytes for the Mobile Phase

For ESI and APCI ion sources, the solvent and electrolytes must be volatile and polar. APCI solvents must also be protic.

**Acceptable Solvents for ESI:** acetonitrile, methanol, tetrahydrofuran, water

Pure acetonitrile or pure tetrahydrofuran are not effective solvents for ESI+, because there is no acidic proton to transfer to the analyte. A small amount (0.1%) of formic acid or acetic acid is often added to acetonitrile or tetrahydrofuran to provide the necessary acidity.

**Acceptable Solvents for APCI:** methanol, water

Do NOT use acetonitrile when using APCI. Acetonitrile is not a good reagent compound for chemical ionization, and it burns in the APCI corona leaving carbon deposits. A water/methanol mixture is a good solvent choice for APCI.

### Acceptable Electrolytes:

Acidic Conditions: formic acid, acetic acid

The pH should not drop below 2. Aqueous 0.1% HCOOH is near pH 2.6 .

Near Neutral Conditions: ammonium formate, ammonium acetate

Aqueous NH<sub>4</sub>OAc is near pH 7.0 .

Alkaline Conditions: ammonia

The pH should not exceed 10. Make sure the column can withstand the mobile phase pH. Silica begins dissolving above pH 9.

Electrolyte concentrations should not exceed 0.1% (w/v) or 10 mM

Contact Dr. Blauch if you are considering using other solvents.

<b>Unacceptable Solvents:</b>	DMF, DMA, DMSO	any high boiling solvent
	Hexane, Toluene	any hydrocarbon
	Triethylamine	any amine
	CH <sub>2</sub> Cl <sub>2</sub> , CHCl <sub>3</sub>	any halogenated solvent

## Solvent Preparation

All solvents and electrolytes must be LC-MS grade. Do not use lower purity solvents.

Water must be freshly purified Type I grade.

Aqueous solutions should be stored in an amber bottle to minimize exposure to light, which accelerates microbial growth. Add a drop or two of acetonitrile to aqueous solutions to deter alga growth.

All glassware coming in contact with the solvents must be meticulously clean.

Do NOT use detergents or other substances that might leave residues on the glassware as these can contaminate the LC and the mass spectrometer.

A special set of glassware should be set aside for exclusive use in preparing LC-MS solvents.

This glassware should be brand new (never exposed to detergents or other reagents) and should be stored upside-down to prevent the accumulation of dust particles. The glassware should be thoroughly rinsed with fresh deionized water followed by LC-MS grade methanol or isopropanol. Do not use this glassware for any other purpose.

Glassware specially designated for preparing LC-MS solvents is available in the white tub near the instrument. Use only the solvent bottles in this white tub (amber for predominantly aqueous solvents, clear for organic solvents). Always thoroughly clean solvent bottles and return them to the white tub. Never use any other bottles for LC-MS solvents.

If salts are dissolved in the solvent or an unfiltered stock has been used, the solvent must be filtered (0.22  $\mu\text{m}$ ). Contact Dr. Blauch for instruction on filtering solvents.

Prepare only sufficient solvent for your immediate needs. Aqueous solutions should be replaced every day or two. Organic solvents should be replaced weekly. Acetonitrile is prone to polymerize. It is best to place 95% acetonitrile/5% water rather than pure acetonitrile in the reservoir. Unless you plan to perform extensive experiments, do not prepare more than 200 mL of mobile phase.

When preparing fresh mobile phase, **discard the old mobile phase** (into the waste container). Do not add fresh mobile phase to old mobile phase. Always prepare a new batch of solvent and place it in a clean bottle.

If your group uses the LC-MS heavily, it should purchase its own LC-MS solvents.

## Sample Preparation

1. All samples must be fully dissolved and be free of particles. Ideally samples would be centrifuged for 10 minutes. Draw off the top portion of the liquid and transfer it to an LC-MS sample vial for analysis. A syringe filter (0.2  $\mu\text{m}$ ) may be used, but syringe filters and plastic syringes introduce contaminants into samples.
2. The solvent and any electrolytes must be compatible with the LC-MS (see previous section on **Solvents and Electrolytes for the Mobile Phase**).

If possible, avoid phosphate, borate and other good bases in your samples. In positive ion mode, any compound that is more basic than the analyte will suppress protonation of the analyte, leading to significant loss of signal. Phosphate and borate compounds can remain in the ion source for many minutes, so it is best to avoid introducing these ions in the ion source. If phosphate or borate is present, chromatography should be used to separate these ions (usually at the very beginning of a reverse-phase separation). The mobile phase containing the phosphate and borate should be diverted to waste rather than into the ion source.

3. The QTOF mass spectrometer is highly sensitive and easily overloaded. A reasonable starting concentration is 1 mg/L or 1  $\mu$ M.

**Start with a low concentration.** If no analyte ions are observed, increase the concentration or the amount injected. Ionic compounds and easily ionized samples can give strong signals at concentrations well below 1  $\mu$ M. If you have difficulty seeing a signal for your compound, please contact Dr. Blauch for assistance.

## Methods

Each application requires an acquisition method specifically developed to address the unique features of the analyte and matrix and the desired information. Contact Dr. Blauch for assistance in developing methods for your samples.

### Pump Settings

If a gradient separation is performed, be sure to allow sufficient post-run time for the column to be flushed and equilibrated with the starting mobile phase before the next run begins. The dead volume from when the composition of the mobile phase is changed until the new mobile phase reaches the mass spectrometer is about 1.3 mL. If the flow rate is 0.30 mL/min, it will take 4.3 min for the new mobile phase to reach the mass spectrometer. Allow a few extra minutes to allow the column to equilibrate with the new mobile phase. In this example, a post-run time of 8 min would be reasonable.

### Typical QTOF Method Settings

The table below provides appropriate ESI ion source settings, which depend upon the mobile phase flow rate.

Flow Rate (mL/min)	Nebulizer Pressure (psig)	Drying Gas Flow Rate (L/min)	Drying Gas Temperature (°C)
0.05	20	10	325
0.2	40	12	340
0.3	40	12	340
0.5	45	12	340
1.0	50	12	350

Note: Do not operate at 1.0 mL/min if the mobile phase is mostly aqueous.

### **Fragmenter Voltage**

Start at 150 V and vary the fragmenter voltage in 30 V increments (up and down) to maximize signal. If the mass spectrum shows adducts with calibrant or other compounds in the solution, try increasing the fragmenter voltage to break up the adducts. If the analyte is easily fragmented, try lowering the fragmenter voltage.

### **Capillary Voltage**

For ESI+, the capillary voltage is usually between 3500 and 4500 V.

For ESI-, the capillary voltage is usually between 3000 and 3500 V.

Vary the capillary voltage in increments of 300 V to maximize the signal.

Increasing the capillary voltage tends to enhance the signal for high m/z ions more than for small m/z ions.

## System Operation

### I. Inspect the Instrument

#### Summary

Before starting an experiment, determine whether the instrument is configured to perform the desired experiment.

1. Open the log file ([LCMS-Log.xlsx](#) on the Desktop) and sign in on the User sheet for the current year.
2. Read the recent log entries and inspect the instrument components.

#### **Is the correct column installed?**

If necessary, change the column. [Document the column in the log file.](#)

[If you change the column, be careful not to pinch the PEEK tubing at the edge of the column compartment!](#)

Contact Dr. Blauch if you are unsure how to change the column.

#### **Are sufficient quantities of the correct solvents available?**

If necessary, replace or change the solvents. Be prepared to make your own solvents.

All reservoir bottles must be [clearly labeled](#).

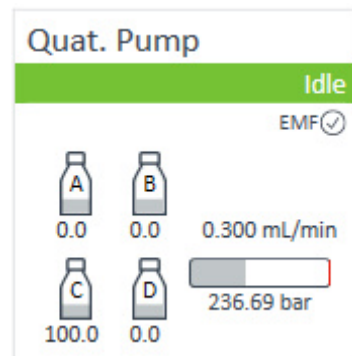
Make sure Reservoir C contains adequate 80% MeOH. You will need to flush the LC with this solvent at the end of your experiments.

Make sure the Seal Wash bottle contains adequate 10% Isopropanol.

[Discard old mobile phase and wash the bottle with fresh DI water and then pure solvent before refilling the solvent bottle.](#)

Double-click on the appropriate bottle in the Pump module and update the amount of solvent in the bottle. The acquisition software tracks solvent usage. If the program thinks a reservoir is empty, it will shut down the pump and terminate the experiment.

Contact Dr. Blauch if you are unsure how to change the solvents.

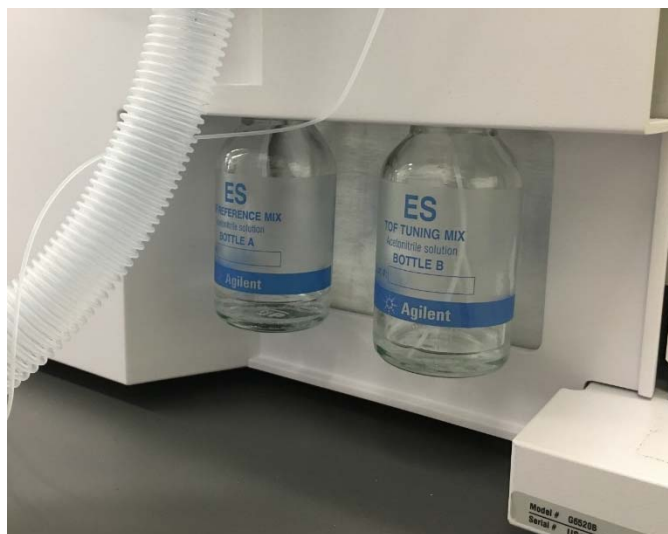


**Is there sufficient Calibrant A and B on the QTOF?** (See picture below.)

Calibrant A (on the left side) is introduced into the ion source with the mobile phase to provide internal standards for mass calibration.

Calibrant B (on the right side) is used to calibrate the instrument before running experiments.

Note: Different calibrants are used for ESI+, ESI-, and APCI. Make sure the correct calibrant is in place.



Contact Dr. Blauch if more calibrant is required or the calibrant needs to be changed.

**Is the correct ion source (ESI or APCI) installed?**

The current source should be identified in the log file. The source is also specified under “Ion Source” in the Tune Context (see below).

Contact Dr. Blauch to change the ion source.

3. Open the MassHunter Acquisition Program (it may already be open) and check the QTOF pressures, which are shown in the upper right corner of the window.

The rough pump pressure should be < 2.5 Torr

The turbo pump 1 (Q) pressure should be < 20  $\mu$ Torr

The turbo pump 2 (TOF) pressure should be < 0.5  $\mu$ Torr

If any of these pressures is high, contact Dr. Blauch and do not turn on the QTOF.

Parameter	Value
Q-TOF: Not Ready Text Short	
Q-TOF: Rough Vac	1.87E+00 Torr
Q-TOF: Quad Vac	1.49E-05 Torr
Q-TOF: TOF Vac	2.52E-07 Torr
Q-TOF: Chamber	3.61 $\mu$ A
Q-TOF: Capillary	4.498 $\mu$ A
Q-TOF: Ref. Mass Ions Found	All_Ions_Found
Quat. Pump: Ripple	0.82 %

Record the pressures in the log file.

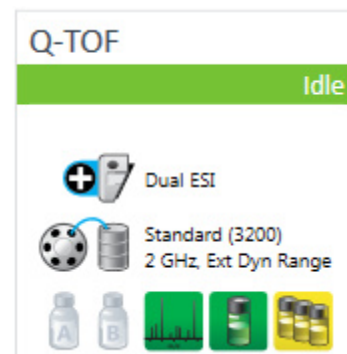
## II. Prepare the Instrument

### Summary

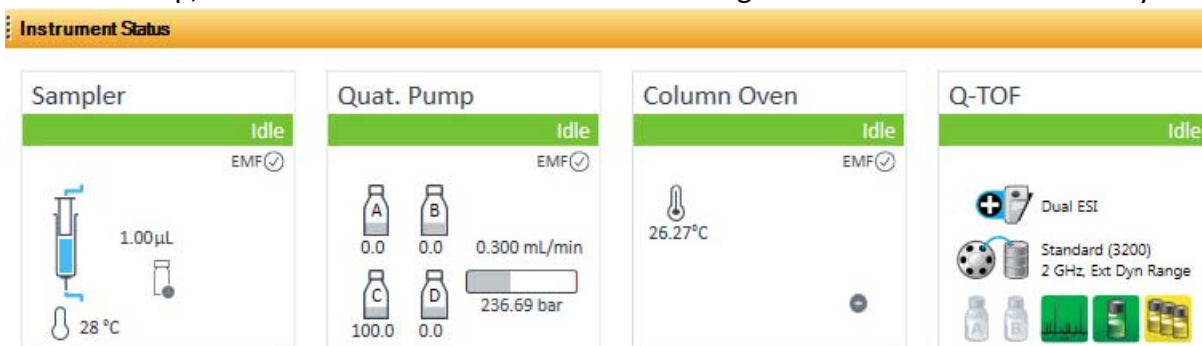
Turn on the QTOF and start the LC pump.

1. If the QTOF is in Standby, turn on the QTOF by right-clicking in the QTOF control window and selecting "On". After a minute or two, the green **Idle** status should appear.

Agilent recommends the QTOF be "on" for at least 30 min prior to calibration and use. While the QTOF is stabilizing, prepare the LC.

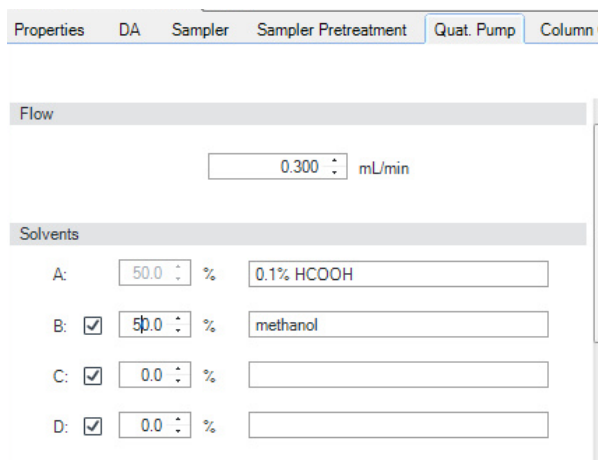
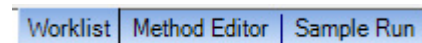


2. In the acquisition program, right-click in the white area and turn on all LC components: Sampler, Quat. Pump, and Column Oven. The status should be green and read **Idle** when ready for use.



3. If a solvent reservoir has been replenished, it will be necessary to purge the lines to remove air from the LC lines. If the LC has been idle for several days or longer, it will probably be necessary to purge the lines.

If the system had been idle, the system should be running the **Idle** method. If necessary, load this method. (Never save any changes to this method.) At the far lower left corner of the Acq window are three tabs. Select the **Method Editor** tab.

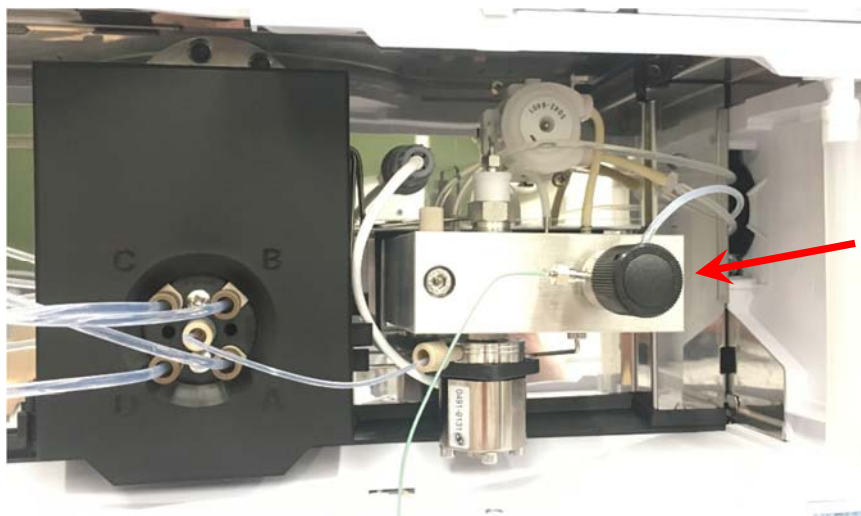


In the **Quat Pump** section of the LC-MS method, set the pump to draw equal percentages from the reservoirs involved in the purge. In this example, Reservoirs A and B are to be purged.

At this point, the flow rate should be a normal operating value (0.1 to 0.5 mL/min).



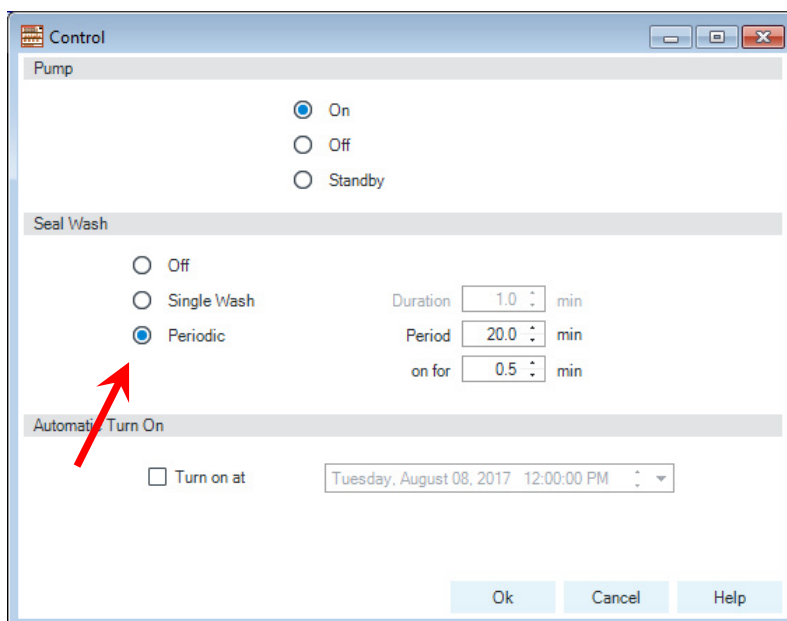
Next locate the pump itself. The pump is the lowest module in the LC stack. Open the right door to the pump and open the black purge valve on the right side of the unit. Opening this valve diverts the flow to waste instead of to the column.



In the Quat Pump method, set the flow rate to 4 mL/min. Maintain this rate for **4 min for each reservoir** supplying solvent. (If you are using two reservoirs, A and B in this example, then allow the purge to run for 8 min.) The high flow rate will flush air from the lines between the reservoirs and the sampler.

When finished, set the flow rate back to a normal operating value (*e.g.*, 0.1 to 0.5 mL/min) and close the purge valve.

4. **Turn on the Seal Wash** by right-clicking in the Quat. Pump control window. Select the **Periodic** Setting under the Seal Wash category.



5. **Load your method**, which should change the solvent to the desired starting composition and the desired flow rate. It will take at least 10 min to flush the system with your starting mobile phase. During this time, it is best to divert the LC flow to waste. Find the Q-TOF tab in the method. Under the LC Stream option, select Waste and click Apply Now.

The screenshot shows the Q-TOF software interface. At the top, there are tabs for 'Column Oven' and 'Q-TOF'. Below these are sub-tabs: 'General', 'Source', 'Acquisition', 'Ref Mass', and 'Chromatogram'. The 'General' sub-tab is selected. It contains several sections: 'Ion Polarity (Seg)' with radio buttons for 'Positive' (selected) and 'Negative', and a checkbox for 'Fast Polarity Switching'; 'LC Stream (Seg)' with radio buttons for 'MS' and 'Waste' (selected), and an 'Apply Now' button; 'Data Storage (Seg)' with radio buttons for 'None', 'Centroid', 'Both' (selected), and 'Profile'; and two threshold sections. The 'Plot and Centroid Data Storage Threshold' section has two columns: 'MS' with 'Abs. threshold' (200) and 'Rel. threshold (%)' (0.01), and 'MS/MS' with 'Abs. threshold' (5) and 'Rel. threshold (%)' (0.01). The 'Profile Data Storage Threshold' section has 'MS threshold' (0) and 'MS/MS threshold' (0).

**Monitor the pump pressure** when your mobile phase is being pumped through the system. The red trace in the Chromatogram Plot should show the pressure. The pressure may also be found in the Quat. Pump control window in the upper left of the Acq window.

The maximum pressure for the 1260 pump and the house column is 600 bar.

If necessary, lower the flow rate to make sure this limit is not exceeded.

Pump mobile phase through the system until the LC pressure is stable for several minutes.

Note: It would be prudent to flush the column with “good” mobile phase (acetonitrile or methanol for a reverse-phase column) for 10 minutes or longer to clean contaminants off the column.

If the column was changed (in Step I-2), check the column connections to make sure there are no leaks.

### III. Calibrate the QTOF

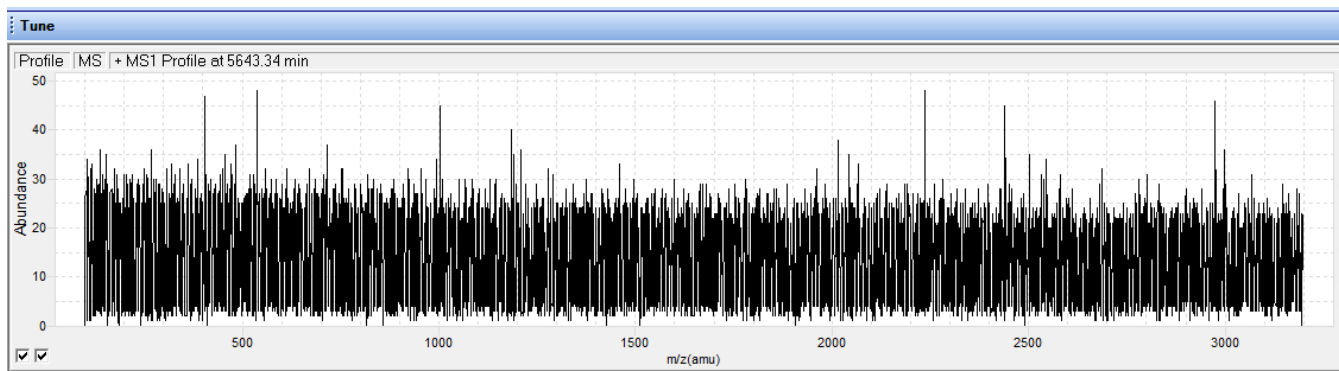
#### Summary

Select the desired operating mode for the QTOF and, if necessary, calibrate the QTOF.

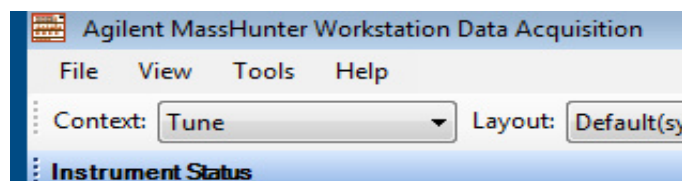
The QTOF must be calibrated daily. [Check the log file](#). If the instrument was calibrated earlier in the day, you do not need to repeat this step. Otherwise, follow the steps below. Separate calibrations are required for positive and negative ion modes.

1. The QTOF should be “On” for at least 30 minute before performing the calibration.

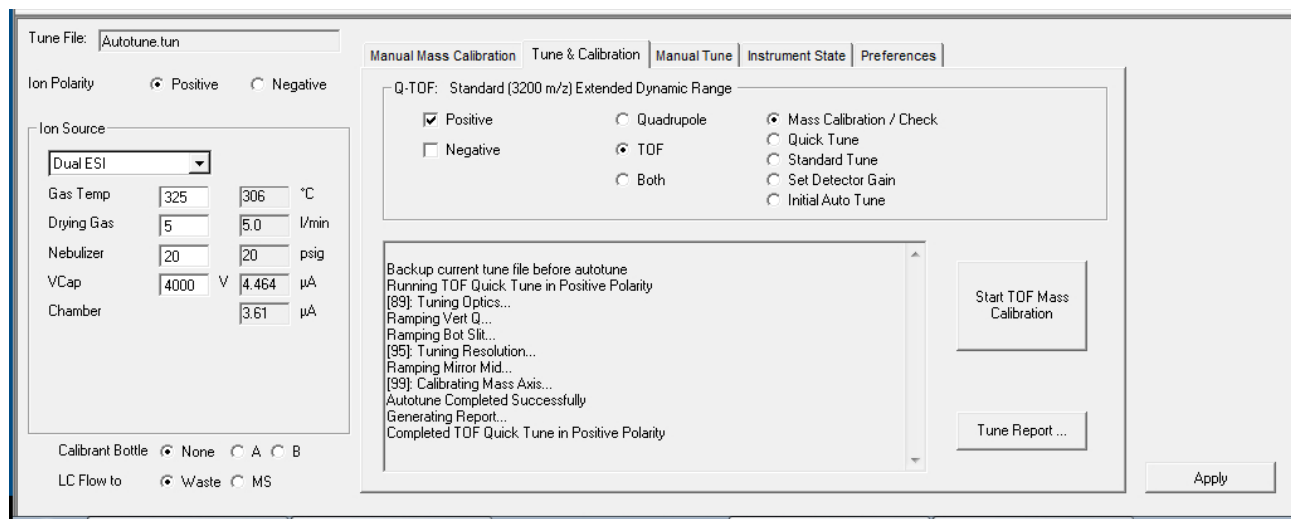
You should see baseline noise that is generally < 30 counts but with occasional higher intensity spikes, as shown below. None of the higher intensity spikes should be persistent.



2. In the Acquisition program, select the [Tune](#) context in the upper left of the acquisition window.



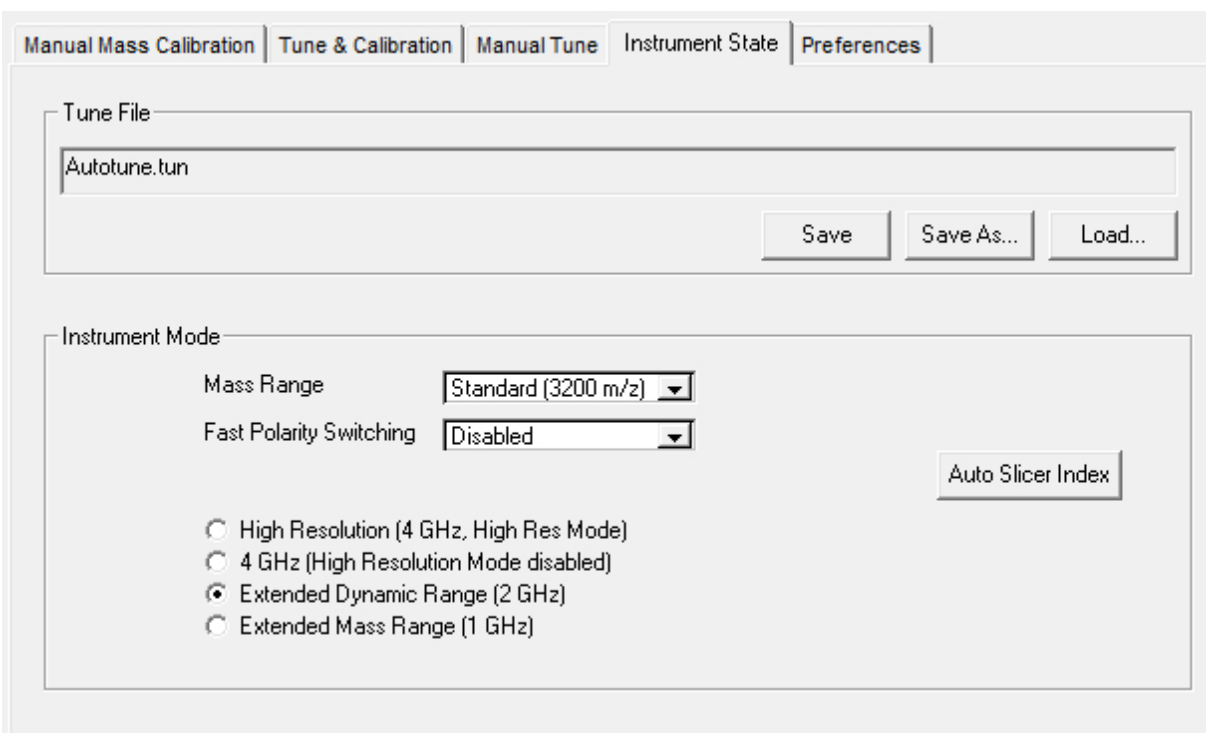
You should see the tune box shown at the top of the next page.



In the upper left corner, note that the ion source (**Dual ESI**) is shown at the left and the Ion Polarity is **Positive**. Make sure the LC flow is going to waste (lower left corner).

3. Verify that the instrument is in **Standard (3200 m/z)** mode with the **Extended Dynamic Range (2 GHz)**. The state is written across the top of the above image, just below the tab.

If it is necessary to change the mode, go to the **Instrument State** tab and select the desired mode. After any change in the mode, the QTOF requires 20 minutes to stabilize in the new mode.



4. In the Tune and Calibration tab, select **Positive** ion mode and the **TOF** as well as the **Mass Calibration/Check** option, as shown on the next page.

Manual Mass Calibration | Tune & Calibration | Manual Tune | Instrument State | Preferences

Q-TOF: Standard (3200 m/z) Extended Dynamic Range

☒ Positive ☐ Negative ☐ Quadrupole ☒ TOF ☐ Both

☒ Mass Calibration / Check ☐ Quick Tune ☐ Standard Tune ☐ Set Detector Gain ☐ Initial Auto Tune

Backup current tune file before autotune  
Running TOF Quick Tune in Positive Polarity  
[89]: Tuning Optics...  
Ramping Vert Q...  
Ramping Bot Slit...  
[95]: Tuning Resolution...  
Ramping Mirror Mid...  
[99]: Calibrating Mass Axis...  
Autotune Completed Successfully  
Generating Report...  
Completed TOF Quick Tune in Positive Polarity

Start TOF Mass Calibration

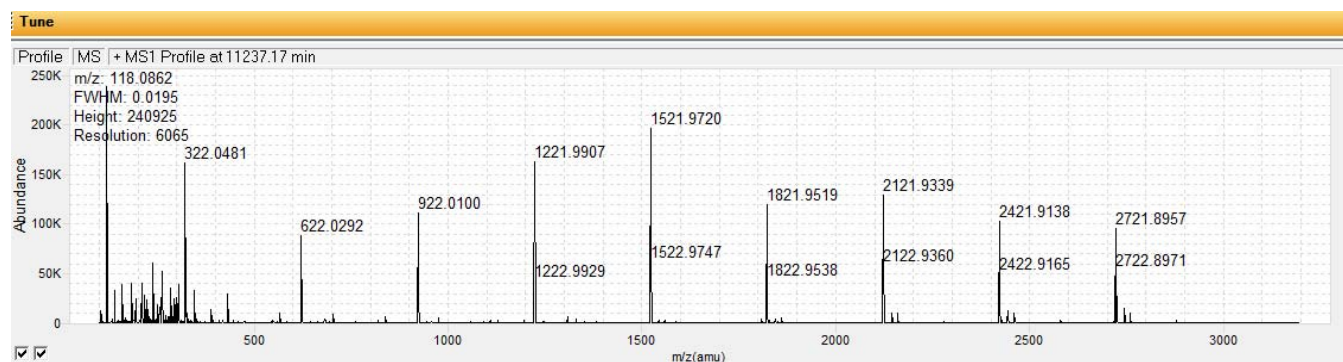
Tune Report ...

5. Turn on **Calibrant Bottle B** (instead of "None").

Calibrant Bottle ☒ None ☐ A ☐ B

LC Flow to ☒ Waste ☐ MS

After a minute or two, the QTOF status should turn green (**Idle**) and you should see the spectrum shown below. If you do not see a spectrum similar to this, contact Dr. Blauch.

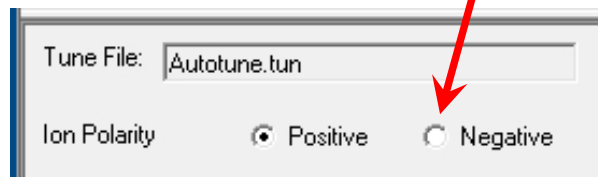


6. Click on **Start TOF Mass Calibration** to perform the calibration, which should only take a few minutes.

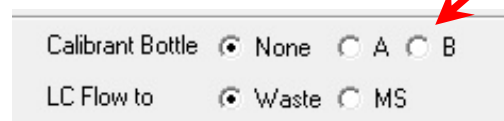
When calibration is complete, the instrument will automatically turn off the calibrant, divert the LC flow to the mass spectrometer, and display the calibration report.

7. Examine the tune report (which is automatically displayed) for the following items:
  - a. Residuals for all calibrants should be within 0.3 ppm of the calibration curve.
  - b. The resolving power for the 2722 ion should be > 14,000.Contact Dr. Blauch if either of these results lies outside these limits.

8. For negative ion experiments, select the **Negative** setting under **Ion Polarity** at the far left of the screen.



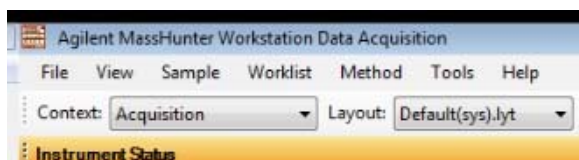
Divert the LC flow to **Waste**, and then turn on **Calibrant Bottle B**, as before.



In the Tune and Calibration tab, select **Negative** ion mode, **TOF** and **Mass Calibration/Check** option, and start the calibration, as described above. When the calibration is complete, check the residuals and resolution in the calibration report.

9. When calibration is complete, the instrument will automatically turn off the calibrant and divert the LC flow to the mass spectrometer.

Change the context to Acquisition, being sure to [save the tune file](#) when prompted.



## IV. Sample Analysis

### Summary

Assuming you have already developed a method for your analysis, you are now ready to run your samples.

1. Your experimental method should be loaded (from Step II-4) and the column should be equilibrated with your starting mobile phase. If you are performing a new analysis for which no method exists, contact Dr. Blauch for assistance in method development.

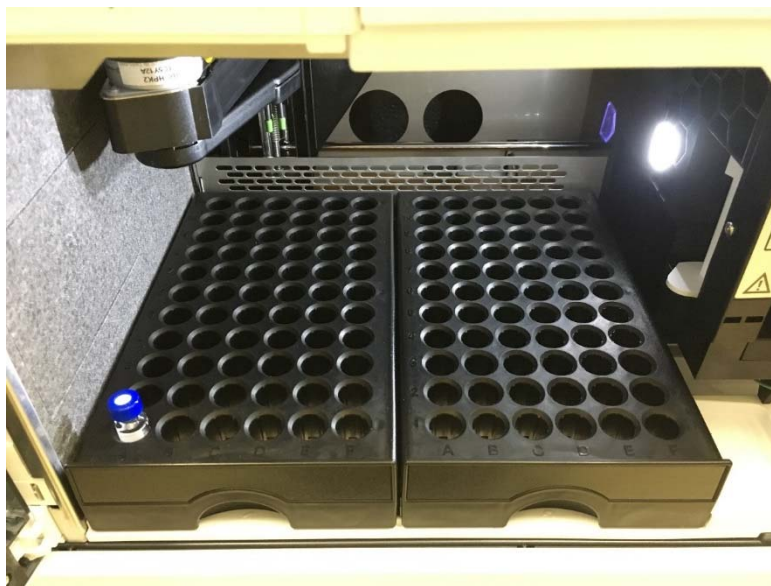
Be attentive to the following items in the acquisition method:

- a. In the Quat. Pump control, make sure the reservoir levels reflect the actual content of the reservoirs. The Quat. Pump should be set to shut down the LC if it thinks a reservoir is empty in order to prevent damage to the pump.
  - b. Check the pressure limit for the column and enter this value in the pump method. The house column has a pressure limit of 600 bar. Set a minimum pressure of 5 bar to stop the pump if a major leak occurs.
  - c. For gradient experiments, provide an adequate post-time to allow the column to re-equilibrate at the initial conditions before the next injection. (See the information on the Pump Settings on page 11.)
  - d. At the end of an experiment, the chromatographic mobile phase program should run "good" solvent through the column for a sufficiently long time to remove all compounds introduced onto the column during the experiment. Five to ten minutes is typical.
  - e. Accurate quantitation requires at least 12 points across a peak. If you plan to integrate a chromatogram, examine the peak width and determine the appropriate sample rate (spectra/sec). Do not over sample. More than 20 or 25 points across a peak reduces signal-to-noise and can create jagged chromatographic peaks. If you require only qualitative data, three or four points on a peak is sufficient.
  - f. LC-MS data files can be enormous (hundreds of MB or more). To minimize the file size, acquire only the mass range that is required and do not acquire data during segments when the mass spectrum is not needed. If you do not plan to analyze the profile data, collect only centroid data.
2. Monitor the pump pressure and QTOF signal.  
The pump pressure and the signal from the QTOF should be stable (not drifting) prior to starting an experiment. If either property is drifting significantly with time, the column is not equilibrated with the mobile phase or there is a problem with the ion source.

3. Place the samples in the auto-sampler compartment, making a note of their positions.

The left tray is P1; the right tray is P2. Each position in a tray is identified by its letter and number, as marked on the tray.

A sample in the far front left corner of the auto-sampler (as shown in the figure) is in position P1A1.

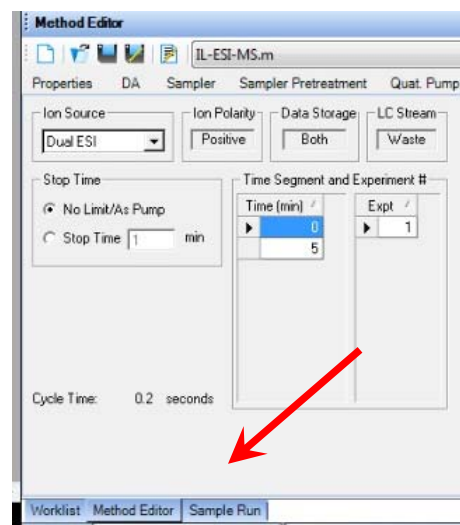


4. Use the Sample Tab to run samples one at a time. Use the Worklist tab to schedule a series of experiments to run automatically.

These tabs are located in the bottom left corner of the window in the Acquisition context.

Be sure to specify your group's data folder (under D:\MassHunter\Data).

Inject small amounts of dilute sample initially in order to avoid overloading and contaminating the LC and the ion source. Increase the injection volume and/or sample concentration as necessary to observe an adequate signal intensity.



5. Monitor the pump pressure during your experiments. The pressure should be constant for isocratic runs and should change reproducibly for gradient runs. Fluctuations in the pressure or variability in the pressure profile from one experiment to the next indicate a problem with the LC.

During the injection, the pressure of the system will sharply drop. This drop should not be less than ca. 40% of the original pressure and should recover very quickly. If the pressure drops to or near zero and/or takes substantial time to recover, there is an air bubble in the injector. Contact Dr. Blauch for assistance in removing the air bubble.



## V. Shutdown

### Summary

After your measurements are finished, the column needs to be washed and then flushed with 80% methanol. During this time the ion source is also being flushed. Finally the instrument is put in stand-by mode and the LC modules are turned off.

1. If the QTOF was operated in anything other than 3200/extended dynamic range, return the mass spectrometer to this default setting.
2. During analysis, Reference A is aspirated into the ion source to provide internal corrections to the mass calibration. Reference A will continue to be aspirated into the ion source after your experiments are completed.

To turn off Reference A, right-click in the QTOF control window and [uncheck the Calibrant option](#). Both A and B calibrant bottles should then be gray, as shown at the right.



If the mobile phase contains substantial electrolyte, flush the LC-MS with a “good” solvent that does not contain electrolyte in order to wash electrolyte from the nebulizer and prevent blockage.

3. Flush the column with a “good” solvent to wash contaminants off the column. (For example, on the house C-18 column, use 95% or 100% acetonitrile or methanol.) Flush the column for at least [10 min](#) to clean the column. If your samples contained strongly hydrophobic compounds, longer washes may be needed. If you anticipate substantial large molecules washing off the column, divert the LC flow to waste during the washing process.
4. Load the Idle method ([Idle.m](#) In the main Methods folder). The Idle method will change the mobile phase to 100% Reservoir C (80% methanol/20% water) at a flow rate of 0.300 mL/min. Flush the system for at least [10 min](#) to flush the column with 80% methanol.
5. Divert the LC flow to waste (if you have not already done so), and place the QTOF in [Stand-by](#) mode. (Right-click in the QTOF control box and select stand-by or click on the red control button.)
6. Right-click in the Quat. Pump control box and turn off the Seal Wash.
7. Turn off the Quat. Pump and the Column Oven.  
If the Sampler temperature control was used, turn it off in the Sample control box.

(It is not necessary to turn off the Sampler, as it will turn itself back on eventually. Just make sure it is not set to thermostat the sample compartment.)

If you are using an LC column dedicated to your work, remove your column and insert the house column or restriction capillary in its place.

8. **Complete your entries in the log file**, being sure to note the LC column, mobile phase, and a brief description of your samples.
9. **Take your samples with you when you leave!**

## Calibration Ions

The specific calibration ions in the **Calibrant B** solution for positive and negative ion ESI are listed below.

Traces of these compounds will remain in the ion source for some time and thus will appear in your mass spectrum.

(The ESI+ ions are protonated calibrant molecules. The ESI- ions are trifluoroacetate adducts of the calibrant molecules.)

ESI+	m/z		ESI-	m/z	
	118.0863			112.9856	CF <sub>3</sub> COO <sup>-</sup>
	322.0481			301.9981	
	622.0290			601.9790	
	922.0098			1033.9881	
	1221.9906			1333.9689	
	1521.9715			1633.9498	
	1821.9523			1933.9306	
	2121.9332			2233.9115	
	2421.9140			2533.8923	
	2721.8948			2833.8731	

**Reference A** is typically nebulized during LC-MS experiments to provide real time correction to the calibration in order to maintain 2 ppm mass accuracy. These peaks must be in your mass spectrum in order for the calibration correction to work correctly.

These calibrants might form ion-pairs or adducts with your analytes. It is possible to disable Reference A, if the peaks interfere with an analyte, but it will take a long time to wash all traces of these calibrants from the ion source.

The current **Reference A** contains the following ions. These peaks should be clearly visible in all mass spectra.

ESI+	m/z		ESI-	m/z	
	121.0509	purine + H <sup>+</sup>		112.9856	CF <sub>3</sub> COO <sup>-</sup>
	922.0098			1033.9881	

In ESI-, one might also see the formate (966.0008) and/or acetate (980.0164) adducts of the heavy reference compound: *hexakis*-(1H,1H,3H-tetrafluoropropoxy)phosphazine (C<sub>18</sub>H<sub>18</sub>F<sub>24</sub>N<sub>3</sub>O<sub>6</sub>P<sub>3</sub>, M 921.0025). Reference A also contains ammonium trifluoroacetate, thus one might see ammonium ion and trifluoroacetate (112.9856) ion-pairs.

## Contaminant Ions

The QTOF mass spectrometer is extremely sensitive and can detect trace amounts of compounds. It is important to keep mobile phases as pure as possible to minimize contaminants in the system. Samples should be prepared in a way that minimizes the amount of extraneous compounds in the sample.

Solutions that come in contact with **plastics** may extract **phthalate plasticizers** and polymer fragments.

**Aqueous solutions** always have **red bloom algae**, which release characteristic compounds.

Column bleed peaks are also common.

**Detergents are especially problematic.** Surfactant molecules adsorb on the flow path in the LC and only slow elute. Be sure that none of the glassware used for sample preparation has ever been exposed to detergent. It is best to set aside new glassware for exclusive use in preparing LC-MS samples.

In all circumstances, the mass spectrum will show various ions containing clusters of solvent molecules (*e.g.*, methanol, acetonitrile) around protons, sodium ions, potassium ions, or ammonium ions (ESI+) or around chloride, formate, acetate or trifluoroacetate ions (ESI-).

Often times the cluster ions, contaminant ions, or reference ions are the dominant signals in the mass spectrum.

The Excel log file contains a sheet with contaminant ions (and their sources) that have been identified on our system.