General Thoughts on use of the SCIEX 4000 QTrap Mass Spectrometer
Setup

- Hardware Configuration
    - Configuration of MS with multiple front end components
        - HPLC, UHPLC (multiple vendor options) or trigger switch
        - Source APCI, ESI, Dual Spray, nanoESI – detected via three pin on source for probe
        - Source assembly detection via connection on bulkhead along with HV.
        - These can be built and saved within Analyst under the Hardware configuration tab for activation as needed
- Typical operational parameters
    - Check for vacuum pressure within operational range (10^{-5} – 10^{-7} torr)
        - Good to check roughing pump oil level on site glass periodically
    - Check for status lights of quadrupole
        - Green = good
        - Red = fault status for multiple reasons (pressure, connectivity, HV fault)
    - Check for connectivity between software and hardware components
        - Is source temp online?
        - Is source vacuum venturi ok?
            - No, check waste line for blockage, flush with MeOH if necessary
- Startup
    - Turn on roughing pump (following oil fill) (typically change once per year or less depending on oil grade)
    - After 15 min turn on the mass spec via toggle switch on the end of the instrument.
        - (Though no longer recommended by SCIEX) a small Eppendorf cap or piece of glove can be placed over the opening of the orifice to help speed up the pump down process. If this is done, do not leave it on for extended periods of time (overnight) and also ensure that you plug the waste line below the source attachment point so as to not lose the plug down this line.
    - The instrument will attempt to start up the turbos three times after reaching a vacuum setpoint established by the roughing pumps. If the instrument has not reached an established vacuum setpoint via Turbo startup (both Q1 and Analyzer chamber pumps) after 15 min of pumping, the instrument will autocycle and attempt another restart. This happens three times and then the instrument enters a fault state and must be reset via the toggle switch.
    - Provided the instrument reaches acceptable pumpdown vacuum, continued pumpdown to operational vacuum (10^{-5} – 10^{-7} torr) will continue over the next few hours. Typically operational vacuum can be reached within muliptle hours provided excess solvents are not in the instrument from cleaning, there

is not a leak and the vacuum bulkhead between Q zero and the stubbies is torqued/sealed adequately.
    - During the previous step, activate the desired hardware profile in Analyst
    - Double click on the quadrupole icon in the lower right corner of the software to bring up the status window for pumpdown and coms
- Shutdown of the instrument
    - Reverse the steps for startup. Disconnect the hardware profile, turn off the MS via switch and 15 min later shut off the rough pump.
    - If the instrument "screams" during shutdown, you are venting too quickly and straining the turbos. Over time this can cause failure of the turbo pumps (one of the most expensive components of the instrument).

Startup
CONFIGURE:

- Click Analyst software icon on the desktop. The grey startup window should appear
- Ensure the correct hardware configuration is selected
    - Click Hardware configuration under the configure header on the left user column
    - Select any of the pre-built hardware profiles or build another
    - To build: click new profile, name as desired, click add device. A pop-up menu appears, and you can select device type: Mass spectrometer, pump autosampler, column oven, valve, detector, a/d converter, integrated system and/or software application.
        - Under mass spectrometer select the model – in this case Mass Spectrometer 4000 Q TRAP
        - If tuning, select under pump, syringe pump Harvard
        - If using the nanomate, select the appropriate i/o converter
    - After selecting the profile, click activate profile. A green check should appear to the left of the hardware profile that is selected.
        - If a red X appears to the left of the profile, the system was unable to communicate with the hardware or the profile is built incorrectly for the available hardware. Retrace the build steps and restart Analyst.
        - Occasionally, the hardware will have to be reset manually via the power switches on the MS and the front-end components prior to reactivation of the hardware profile.
    - The reverse is true for deactivating the hardware profile for any shutdown, restart or disconnection needed between software and hardware. This should always be completed prior to power cycling to prevent corrupted files.
        - Shutting down the software during a live data acquisition will generally corrupt the .wiff file.
    - Click close or on the red x to leave the hardware configuration editor

- Security configurations
    - Allows for the administrator to provide access rights for sub users under specific project folders – see “projects” tab, provide users roles within the software for calibration, batch building, review of data, etc, under the “people” tab and setup remote access to the instrument for control of experiments off-site via the “remote viewers” tab.
    - See Figure 1
- Report Template Editor
    - Useful for setting up custom reports for printing (electronic or physical) reports with built in headers/footers
    - Often used with QA/QC documentation of tuning by timestamping and/or electronically signing spectra
- “View Que” button brings up the que list for what is running, what samples are in the que, and which samples are completed. This view can be adjusted by going to:
    - Tools -> settings -> queue options
    - Most useful to adjust is the max # of acquired samples (esp for high throughput)
    - Note: to access tools in the header, you must be under the “configure” substructure on the left column

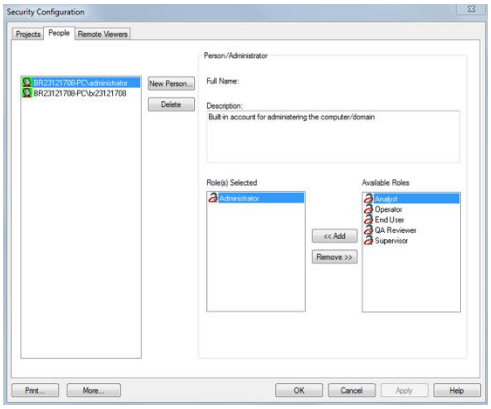


Figure 1: Security Configuration pop-up window for instrument information security control and end-user process/access delineation

TUNE AND CALIBRATE

- Double clicking on “Instrument optimization” allows you a semi-automated means of tuning the mass spectrometer. This is useful if you are wanting to do a quick check of the mass calibration or don’t have time to sit at the instrument and tune by hand.
- Warning: this means of tuning is fraught with errors, as the software will often localize on local min/max values and get stuck in a loop of tuning or fail to complete the task.

MANUAL TUNING:

- Prior to starting any tuning, the instrument must be in “ready” mode. To determine instrument status, the “ready” state is indicated by looking at the quadrupole icon in

the lower right of the software screen. Yellow indicates standby and red indicates a system fault.

- See figure 2

Figure 2: Quadrupole icon for quick reference instrument status identification

- Double clicking on the icon brings up a detailed status window. Items to check include:
    - Status message: if good, the message "operating pressure reached" is displayed. During vent or pumpdown state, this will be indicated in the status window
    - Vacuum gauge: instrument standby is typically $0.9e^{-5}$ torr and when the instrument is activated, is $3\text{-}5e^{-5}$ torr. Note: the instrument cannot be activated unless the vacuum gauge reads $2.0e^{-5}$ torr or less.
    - Backing pump status shows if the roughing pump is on/off
    - Both turbos are indicated here and should read "normal" under the "interface turbo pump" and "analyzer turbo pump" status lines
    - Source/ion path electronics = on
    - And the type of source housing installed will be indicated here. This is dependent on the connection of the source to the bulkhead of the instrument as well as indicated via source circuitry via the probe resistance specific for ESI vs APCI
    - Exhaust pump and interface heater are off for nanoESI. If using UHPLC or some other high flow front end method, ensure the exhaust pumps and interface heaters are enabled so as to not flood the source. The venturi exhaust port should be open.
    - See figure 3

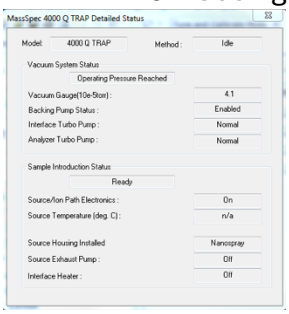


Figure 3: Detailed Status pop-up window for instrument status

- Double click on the "manual tuning" icon on the left column under "tune and calibrate." A generic tune window will pop up. See figure 4
    - File open -> select file type "acquisition method" acquisition method and select file of choice. In this case start with pos mode Q1 .dam file. ->ok
        - Q1Pos_Agilent4000QTrap.dam.

- Using Agilent tune mix as the standard for tuning and calibration a tune file will be generated with 9 masses – 118, 622, 922, 1221, 1521, 1821, 2121, 2421, 2721 da
- The method is set up as an excel sheet where you can right click and add in columns for declustering potential (DP) and entrance potential (EP)
- Mass center, width and time are always present.
- To remove a line simply click on the line and click delect
- Clicking in the upper left corner selects the entire spreadsheet
- Columns can be selected via clicking the header over that column

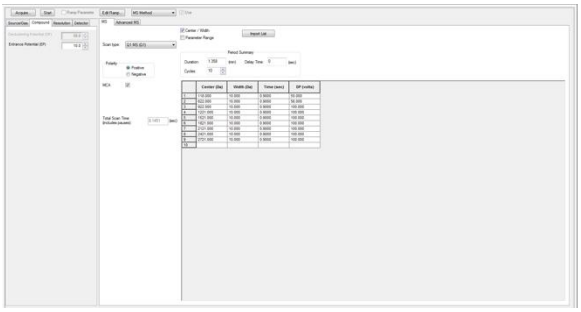


Figure 4: Generic Q1 tune method for Agilent tune mix. Both global and mass specific instrument parameters can be accessed in this method file.

- 4 tabs are located on the upper left side of the active method window – source/gas, compound, resolution and detector. These are global parameters for all masses but are unique to the method generated.
  - Source gas tab item of note: keep the curtain gas as high as possible in order to maximize longevity of the instrument and decrease frequency of cleanings. The curtain gas blows out the curtain plate opening after passing across the orifice plate so the ions are "swimming" upstream from the flow of this gas.
  - Resolution tab item of note: Ion energy (IE1) can be set from 0-2 with "normal operation between ~0.8 and 1.5. The higher this value, the more contaminated the entrance lens on this quad. As the value is increased for signal gain, the instrument is signaling a need for cleaning and/or replacement of the lens via rail pull. Keep this value as low as possible while still maintaining acceptable signal.
  - Detector tab item of note: the CEM detector voltage can be set here but should be maximized to the lowest possible value while still maintaining signal. Rule of thumb: if the TIC doesn't increase by 20-30% when adjusting the CEM voltage by 100 then turn the detector down. Max gain on the detector is 3000 though it will let you go to 3200. There is typically a decrease in detector response above 3000 so not advised to operate at that level. Once the signal gain is no longer achieved at max voltage, the detector must be replaced.
    - This can be accomplished via vent procedure and removal of the detector from the front, right side of the instrument.

- "Edit ramp" tab is useful for selecting the max response of the ion of interest to the ramped parameter. In the case of a Q1 scan the two parameters that can be ramped are DP and EP. Note: EP is typically not ramped and is set across the entire method as 10. However, DP is ion specific. To ramp, delete all but the ion to be ramped, from the method and then select DP in the Edit Ramp window. Set the range (start with a wide range, broad step, for fast ramping and narrow down the range with tighter/smaller steps as you hone in on the tuning signal gain.
- MCA "multiple channel average" check box: select this to average multiple scans. Deselect it when adjusting parameters on the fly otherwise the change will not be observed as it will be averaged into the previous scans.
- Click "start" and two windows will appear – L: TIC and R: MCA. See figure 5.

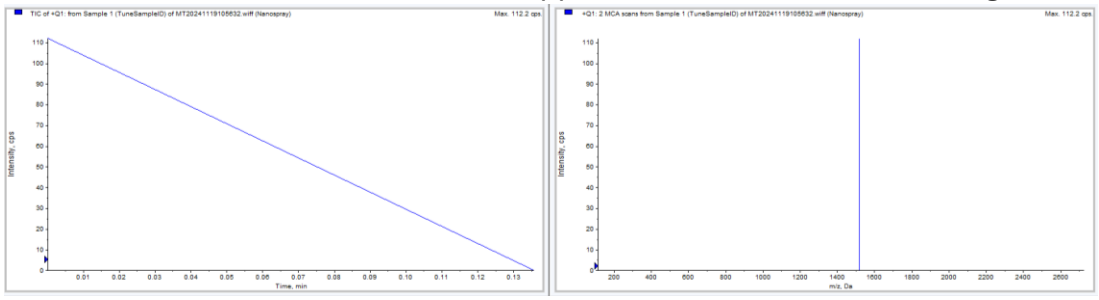


Figure 5: Total Ion Count (TIC) and Multiple/Multi-Channel Average (MCA) windows

  - Use the TIC to monitor infusion response. You want a stable TIC. Instability can indicate air in the lines, pulsing gas (i.e. bad CAD gas solenoids) or improper positioning of the electrode (ESI should be approx. 1-3 mm extended from the probe tip)
  - Right click on the MCA to select the viewing window for each mass of interest
    - Both axes can be zoomed in by selecting outside the trace and dragging
    - Double click on the axes to reset to maximum view
    - Right click -> open file to bring up individual intensity vs m/z windows for all masses of interest
    - Right click on m/z windows to show TIC for the ion or list data
    - List data has three tabs Data list, calibration peak list and peak list
    - All three are able to be viewer customized via right click
    - Note: If the calibration peak list does not appear go to main Analyst header: tools -> settings -> appearance options -> miscellaneous and select "show mass calibration peak list. You will need to reopen the method for this to appear as a tab in the MCA m/z view window.
  - Under the "calibration peak list," the three items to monitor most closely for tuning and calibration are the mass intensity (counts per second – CPS) and the width (da). Finally, the mass shift tells you have far out of calibration your spectrum is. You want this to be less than 0.1 Da. This will be adjusted by updating the calibration curve to a new curve.
  - To update the curve, you must be in an active m/z window (any). Click the Calibrate from spectrum icon in the upper header (5th from right)

        - Under the pop-up window select the standard being used (Agilent), set your search range (keep this as small as possible so to avoid calibrating on incorrect mass peaks inadvertently).
        - See fig 6

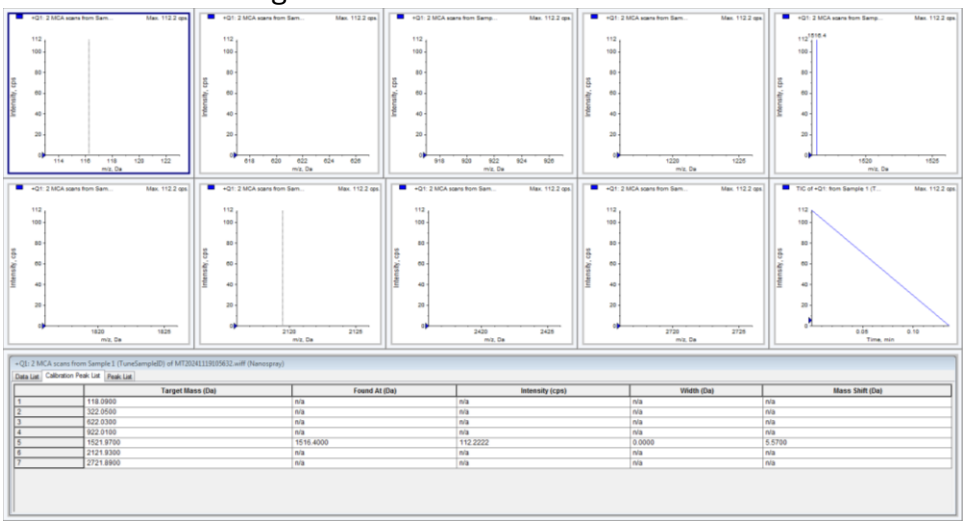


Figure 6: Active m/z window with data list, calibration peak list and peak list information tabulated in a secondary window. Utilize this window for determination of mass width, shift from the calibration curve and intensity during calibration and tuning of the select quadrupole.

    - Click on “replace” or “update” icon to the left of the mass range window and the distribution of masses from calibrated will be displayed.
        - Replace if the entire range should be updated with the new tune parameters
        - Update if only the selected masses should be added/replace the original masses in the tune parameters
- Iteratively repeat this process, adjusting DP, EP, CXP, IE1 and detector voltage until desired mass intensity is reached.
- Adjust the offset for each mass (generally less than 0.01 at a time), update the scan and replace/update the mass calibration.
- Once the final tune has been completed, back up all tune data files externally for safe keeping.
- All tuning files are kept in the following location: D-> analyst data-> API Instrument -> tuning cache. All .wiff files can be reopened in Analyst or PeakView for extracting scan information.
- Note: prior to starting any calibration or tuning, back up the entire API Instrument folder from the D-> Analyst Data-> API instrument location. This can be used to revert the instrument to a previous state if there is any issue with the current tune/calibration process.
- Note 2: The Parameter settings file should be backed up to ensure no file corruption of the original file as all values are written over when tuning and calibrating.
- **For tuning of Q3,** the same process for optimization of signal should be repeated but using a prebuilt Q3 file such as Q3Pos_Agilent4000QTrap.dam.

- Note: IE3 is generally set around 2.0 but can vary and should be established based on general peak shape and intensity found when ramping through the entire voltage range of this parameter.
- Similarly, DP, EP and CXP are ramped parameters. DP and CXP should be set individually for masses while EP is a global parameter set on the lens.

ACQUIRE:

To generate data, you go to the left column of the software, click on either IDA Method wizard (for a step by step process through method building) or "build acquisition method" or "build acquisition batch".

- build acquisition method
  - under the "acquisition method" right click to set the synchronization time and mode with the source if using an external device such as nanomate
  - Under the "mass spec" tab
    - Scan types available include: enhanced MS, enhanced multi-charge, enhanced product ion, enhanced resolution, mrm, ms3, neutral loss, precursor ion, product ion (MS2), Q1 MS, Q1 multiple Ions, Q3 MS, Q3 multiple ions, Time delayed frag
    - Selecting the center width for the mass allows a preset range. Selecting parameter range allows for a unique range for different mass regions in your method
    - See fig 7

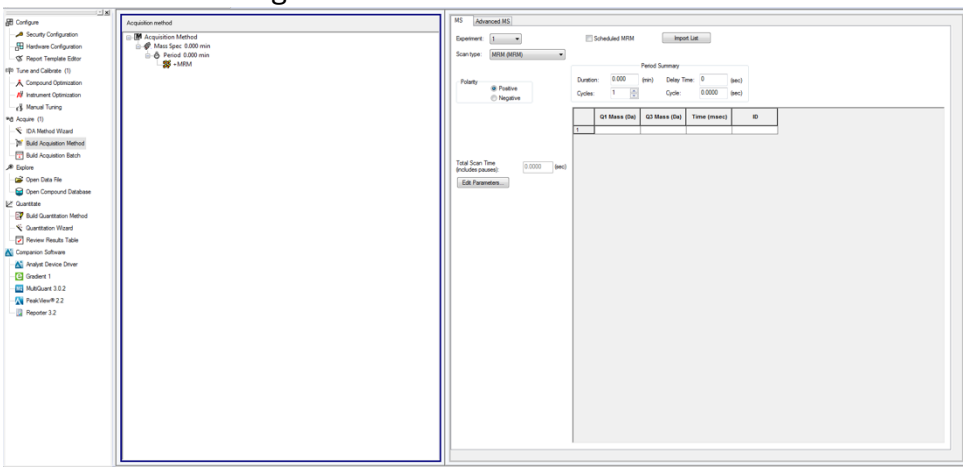


Figure 7: Build Acquisition Method window for method creation.

- Build Acquisition Batch
  - Under the "sample" tab, the Set can be named. This is a submethod where you can have multiple sets under the same master method.
    - See fig 8

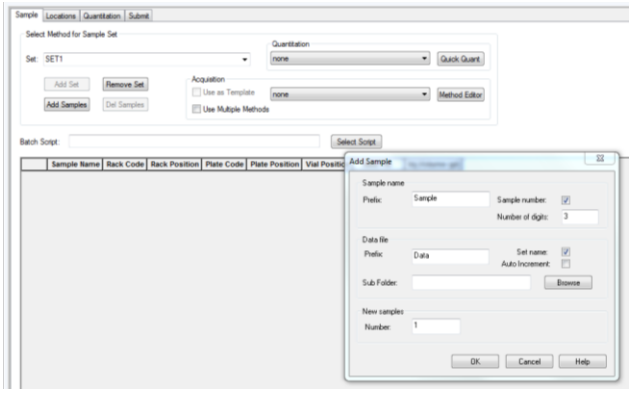
- 

Figure 8: Add Sample pop-up window for creation of batch file name and # of samples.

  - Alternatively, a batch can be built off-line in Excel and imported as a script to the batch build method. Provided all header information is the same, the file will be read to auto populate each column within the build method.
    - See fig 9

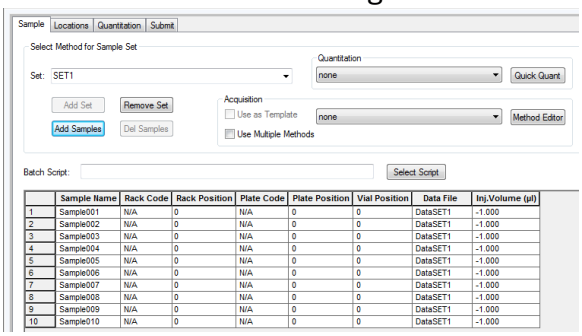
- 

Figure 9: Batch creation can be accomplished through importing a script built off-line or within Analyst.

  - When building out the method, instead of manually typing each item, you can right click on the header and either “fill down” for the same value in the column or “autoincrement” for incremental adjustment of the name (ie sequential nomenclature)
  - Quant types for standard, QC, unknown, Blank, double blank, or solvent
    - See fig 10

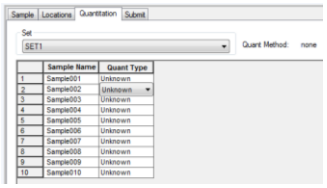
- 

Figure 10: Quantitation tab under the batch creation process allows for selection of QC, standards and other sample types.

  - Submit tab. Can select individual samples for submission or select the entire batch.
    - See fig 11

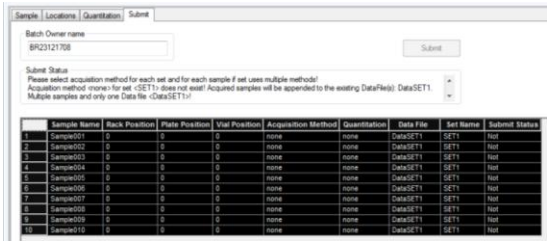
- 

Figure 11: Batch submission can be accomplished via clicking the left upper corner of the data sheet to select all samples or conversely individual selection of a sample and submission is accomplished through highlighting the desired sample

EXPLORE

- Under this tab, there are two options – the first is for simple review of collected data. The second is to explore a database of compounds.

QUANTITATE

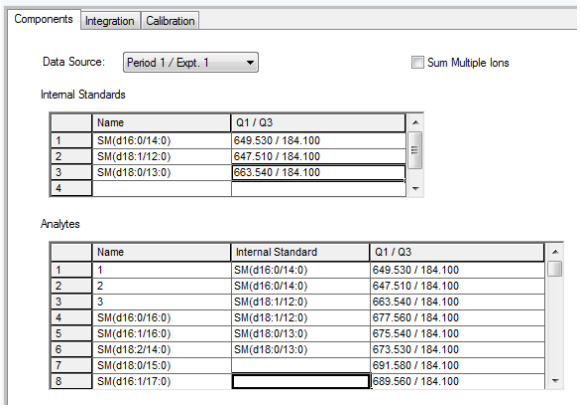
- This tab allows for quantitation method creation and data review utilizing pre-built quantitation methods.
- Under "Build Quantitation Method" the user is able to select a data file previously collected, mark chromatography peaks as internal standards (IS) and indicate elution time (retention time) on a historical chromatogram file. The calibration of future data with this quant method is based on the peak integration of the reference chromatography peaks and the type of calibration curve set for this reference.
    - See fig 12
- 

Figure 12: Quantitation method review window for global build of reference methods for chromatography data review of collected samples.

- Quantitation Wizard is used to for automatic integration of chromatographic peaks. This can be useful for on-the-fly integration, when a quant method is not needed or not available.
- Review Results Table is used for review of integrated data and the associated concentration based on the calibration standards.

Tuning of quadrupoles and MSMS

- Tuning of Q1 with Agilent tuning solution or SCIEX PPG tune solution
- Mass range of the 4000 Qtrap is 3- 2800 Da (slightly higher LMCO for LIT mode)
- Enable the MS hardware profile (no front end) with ESI source

- Ensure a standalone syringe pump is available and run at 10 uL/min 1 mL syringe.
- Connect peek tubing (Red 1/16" OD X 0.03" ID) from syringe to source and start the pump as needed.
- Open a prebuilt method in Analyst (generally labeled Q1). Prior to tune ensure all tune methods are backed up to an external drive in case of hard drive failure.
- Under continuous infusion, scan the entire mass range.
- Generally collect 10 scans avg, right click in TIC and the mass window will open.
- Click on "replace" or "update" icon to the left of the mass range window and the distribution of masses from calibrated will be displayed.
    - Replace if the entire range should be updated with the new tune parameters
    - Update if only the selected masses should be added/replace the original masses in the tune parameters
- Iteratively repeat this process, adjusting DP, EP, CXP, IE1 and detector voltage until desired mass intensity is reached.
    - CEM detector voltage should generally only be increased in increments of 50-100 V and the stability point is where the TIC gain no longer increases 20%+ from the previous voltage setpoint.
- Adjust the offset for each mass (generally less than 0.01 at a time), update the scan and replace/update the mass calibration.
- Once the final tune has been completed, back up all tune data files externally for safe keeping.
    - (POINT TO THIS LOCATION)

- Tuning of Q3
    - Completed in similar manner to Q1, but utilizing a pre-built tune method for Q3.
    - Adjust all tune parameters to ensure desired signal intensity, resolution (0.6-0.8 amu), in this quad.
    - If any global parameters are changed (IQ0, QJet, Detector, etc) return to Q1 tune to establish parameter change functions in this mode as well.

Troubleshooting

- My system will not start
- My m/z spectrum appears to be off by a couple of amu
- I can't hear the roughing pumps on
- I have error status lights on my instrument status panel
- I can't see any ions

Analyst 1.6.3 reference doc:
https://sciex.com/content/dam/SCIEX/pdf/software/an_ref_d1000064246_en.pdf