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).

Tuning

- 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.

Acquisition Method Build

- Building a batch template
- Building a quant template

Data Review

- Analyst
- Peak View

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