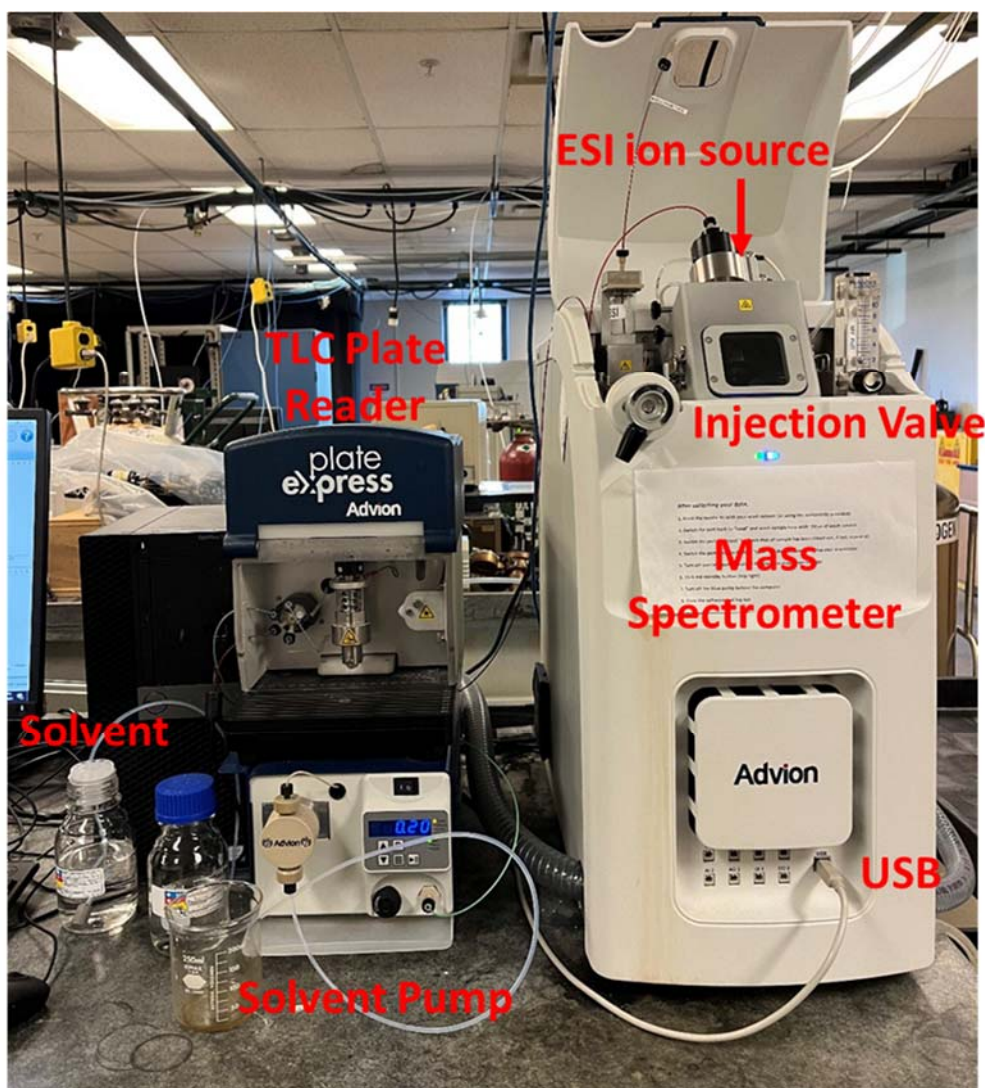


Training Guide for Advion Expression Compact Mass Spectrometer (CMS)

Research Instrumentation Center
Updated 11/30/2021 by Dalton Snyder

The Advion Expression L Compact Mass Spectrometer (CMS) is a single quadrupole mass spectrometer with atmospheric pressure ionization sources capable of analyzing polar and mildly polar compounds at relatively low concentrations ($\sim\text{ppm} = \mu\text{g/mL}$). The Advion is equipped with an isocratic solvent pump, three sample introduction schemes, and two ionization sources and is capable of scanning quickly (10,000 m/z units per s) over a broad mass range from 10 to 2,000 m/z . Samples can be introduced by flow injection analysis (FIA), using the Plate Express TLC plate reader, or using the atmospheric solids analysis probe (ASAP) for solid samples. After injection, gas-phase ions are produced by either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), generally by protonation in positive ion mode or deprotonation in negative ion mode. Both ESI and APCI are capable of ionizing compounds that contain heteroatoms (N, O, S, and P are the most important), but APCI can ionize molecules over a broader polarity range. The Advion CMS is controlled by a simplified user interface which makes it ideal for inexperienced users.



Important consumables to be supplied by user

- Hamilton Syringe (10 μL up to 250 μL)
Needle requirements: 2 inches long, 22 gauge, Point style 3

Sample Requirements

ESI and APCI can generate gas-phase ions from polar compounds. In general, compounds must contain heteroatoms to be ionized and thus be detected by the mass spectrometer. Analytes will usually be detected as protonated or deprotonated ions, i.e. $[\text{M}+\text{H}]^+$ or $[\text{M}-\text{H}]^-$. Sample concentration should be approximately 10 $\mu\text{g}/\text{mL}$ (total amount injected is 50 ng when using the 5 μL sample loop) but may be increased ~ 1 order of magnitude to improve signal-to-noise. **Users should always dilute their samples to low $\mu\text{g}/\text{mL}$ concentration and analyze these more dilute solutions prior to trying more concentrated solutions. Concentrations in the mg/mL range should NEVER be injected.**

Samples should be dissolved in some combination of water, acetonitrile, and methanol, preferably the latter two. Solvents such as THF, DMSO, and nonpolar solvents should not be used. Samples should not contain significant concentrations of buffers, salts, polymers, and inorganic acids and bases.

Acquiring Data (FIA Option)

1. Log in to the computer using the following settings:

User name: .\MassSpecUser

Password: MassSpec1

2. Click on CheMS Express

- a. If you receive a message regarding connection error, unplug the USB connection on the lower panel of the instrument and then reconnect

- b. Log in to your account. If you are a new user, a new account will be set up for you.

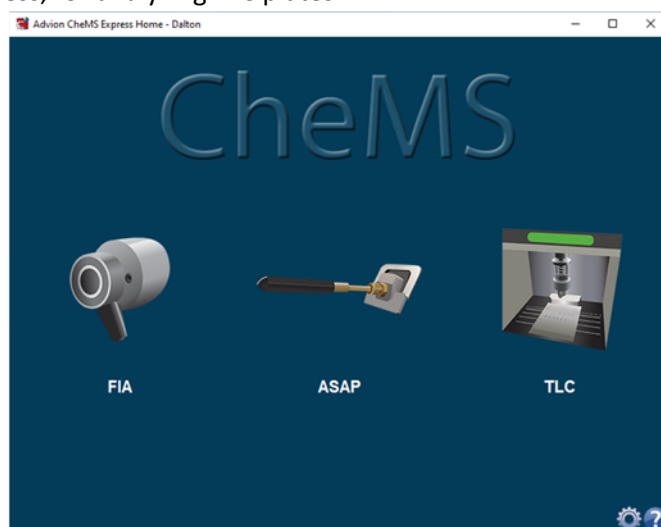
Make sure you write down your password!

3. Three options will appear

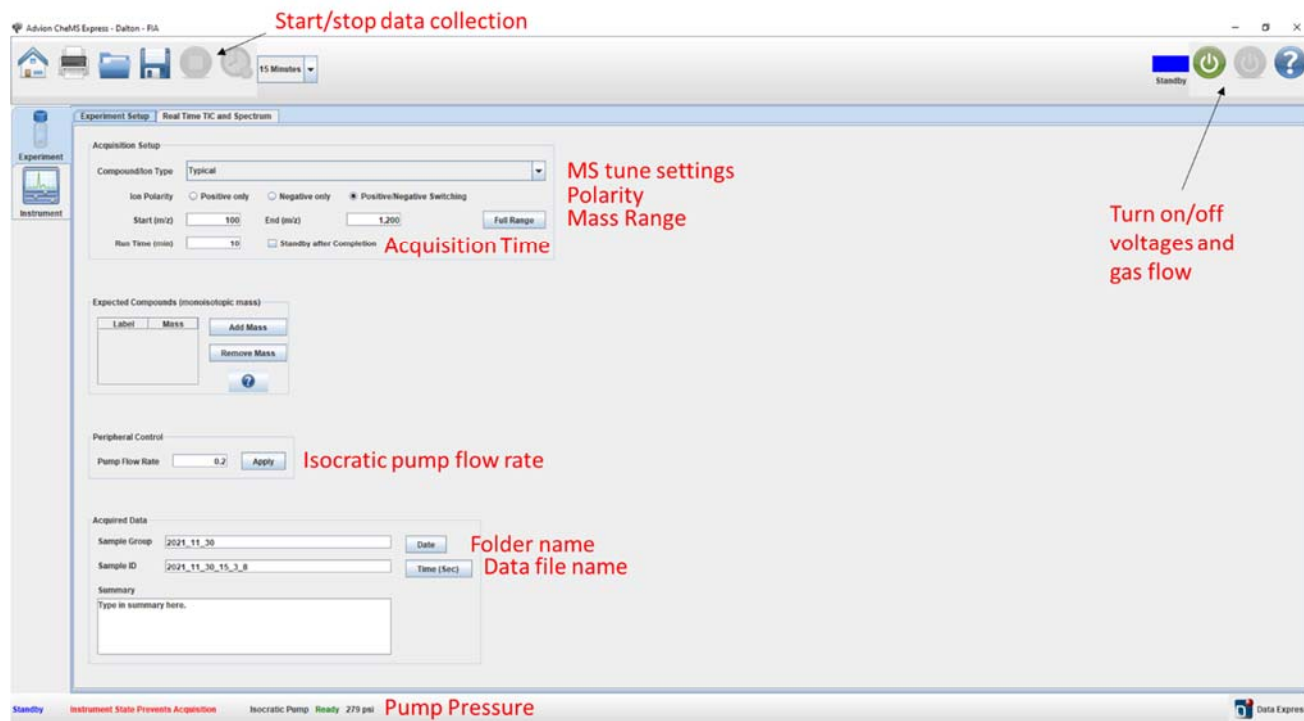
FIA: Flow Injection Analysis: uses the Rheodyne injector on the top left of instrument

ASAP: Solids probe for use with the APCI source

TLC: Uses Plate Express, for analyzing TLC plates



4. Click FIA



5. Click the green button (top right) to switch off standby and turn the voltages and gas flows on.

6. Turn on the blue pump on bench behind the computer. The pump improves sensitivity for high molecular weight species.

7. Turn on the isocratic solvent pump by pushing the play/pause button.

a. At this point the pressure reading displayed at the bottom of the instrument software should increase to the 700 – 900 psi pressure range

b. The pump flow rate can be set either on the pump itself by using the up and down arrow keys or in the software under "Peripheral Control"

c. If the pressure exceeds 2500 psi, turn off the pump and ask for assistance.

d. If the pressure does not increase to the expected range, pause the pump, open the black purge valve by turning it counterclockwise and suck any air bubbles out by using the plastic syringe (check drawers below Advion). Then close the purge valve and turn on the pump.

8. Click on the **Experiment Tab** (far left side of window)

In the **Acquired Data** section at the bottom

Sample Group is a folder generated automatically with today's date

Sample ID is the file name for the data set

Place additional notes in the summary (not required)

In the **Acquisition Setup** section

Select Compound Type (i.e. instrument tune settings, harsh or soft, hot or cold)

Typical is a good starting point but you can try other settings if you want

Select *ion polarity*

Positive, negative, or positive/negative switching

Consider whether your molecule more easily protonates or deprotonates.

Note: 1 data file will be generated per polarity

Input your *Start* and *End m/z* (you should expect to see $[M+H]^+$ or $[M-H]^-$)

Range is 10 – 2000

Input *Run Time*

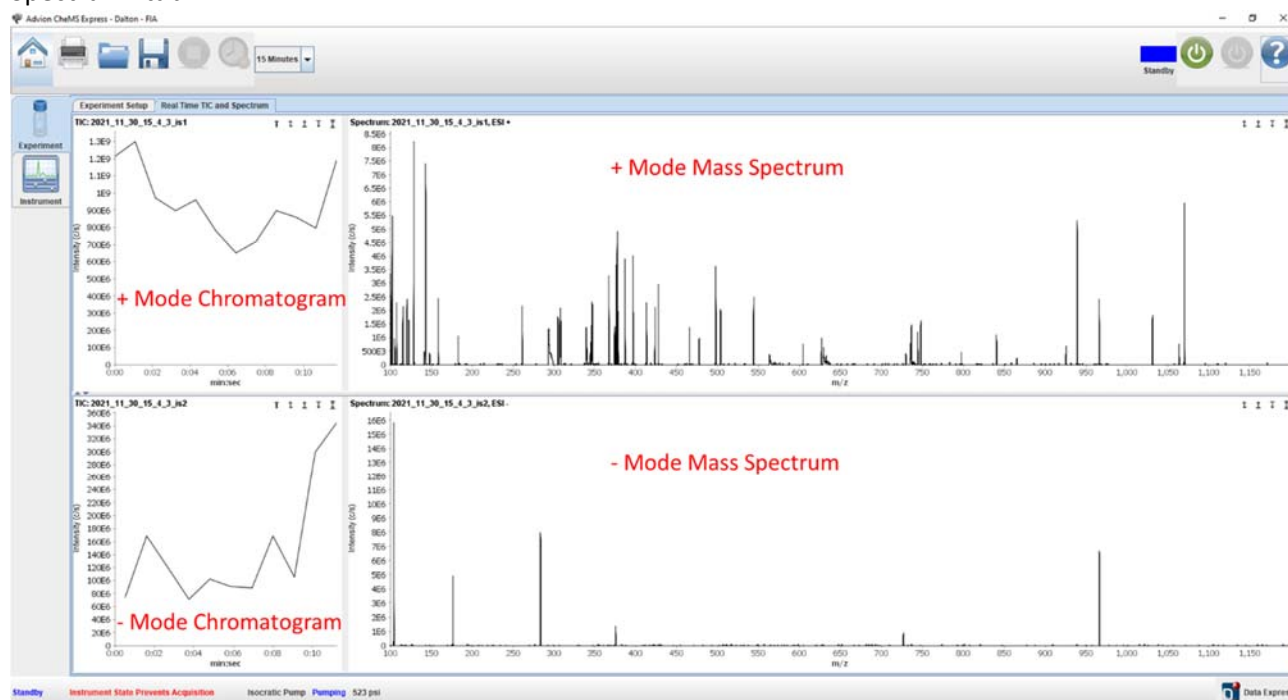
The amount of time you want to collect data

9. Click on the Play/Run Icon (top of screen) to begin acquiring data for the amount of time you specified in the Experiment tab.

10. While the instrument is scanning and collecting data, you need to load and inject your sample:

- Wash your syringe 3x with rinse/blank solution (CH₃CN/Formic acid)
- Load your sample into the syringe
- With the injection port on LOAD (facing ~4-5 o'clock), put the syringe needle into the port and load the sample into the loop (i.e., push the plunger). Pull out the syringe.
- Inject the sample by switching the valve to the INJECT position, pointing at ~7-8 o'clock. Wait a few seconds for the entire sample plug to be pushed through the sample loop and to the ESI source.
- Wash your syringe 3x with solvent.
- You can make multiple injections per data file. Simply repeat the LOAD and INJECT procedure.
- You may want to rinse the sample loop ~3 times between each sample.
- Running solvent blanks is recommended and can help identify background peaks.

11. The total ion count (TIC) and real-time mass spectra can be viewed in the “Real Time TIC and Spectrum” tab.



12. Click on the **Stop** icon when you are done collecting your data files.

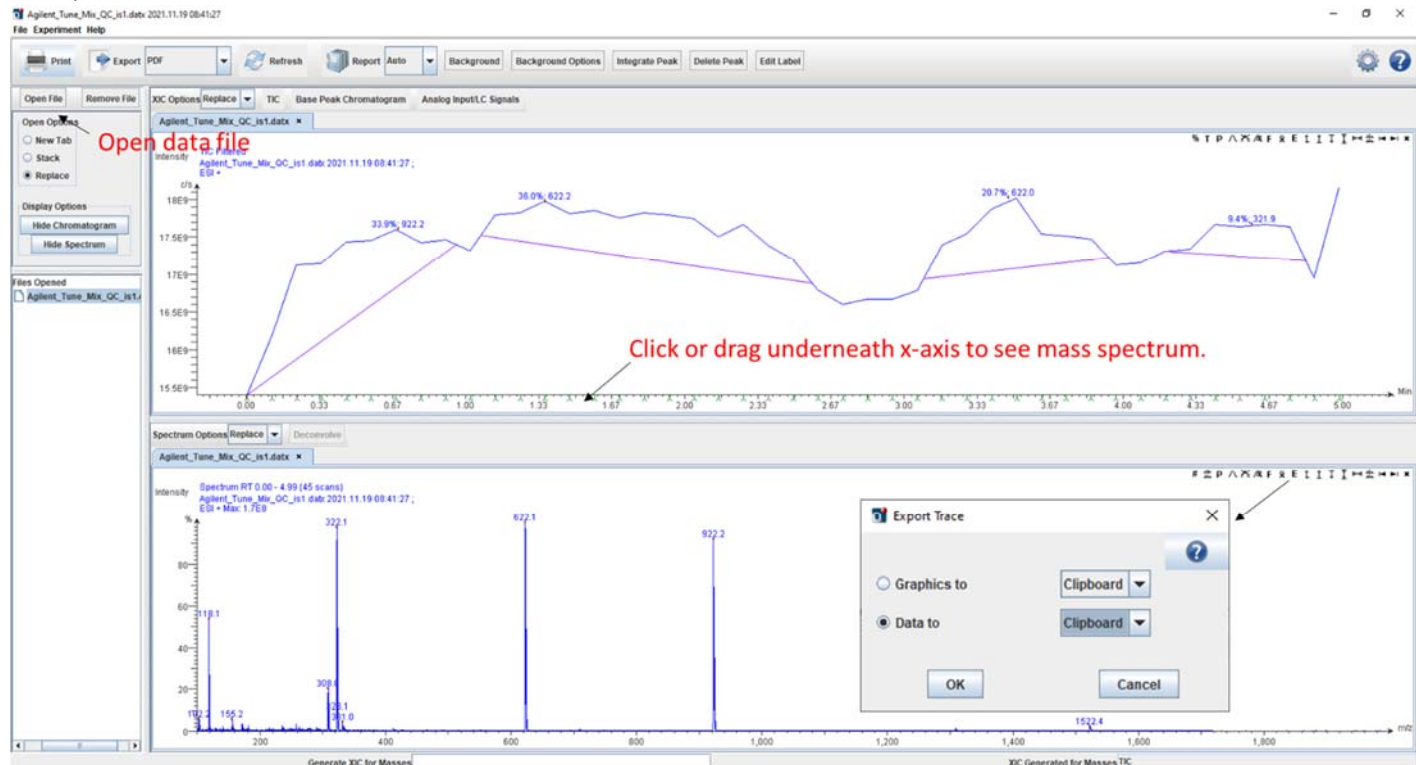
Analyzing Data

1. Click on Data Express (lower right corner in the ChemMS Express software or on the desktop)

2. Open your data file (“Open File”)

- If both positive and negative data were obtained for your sample, there will be two separate data files for each experiment

3. Click and drag on the chromatogram, below the x-axis, to see the average mass spectrum over that time period



Labeling and Exporting Data

Various aspects of the mass spectrum can be manipulated using the small icons in the top right of the mass spectrum. The mass spectrum can also be exported by clicking the the “E” icon, selecting “Data to” and “Clipboard”. Open Excel and paste the data from the clipboard, save the Excel file, and take the data file with you on an external drive (or email it to yourself). You can also export the data as a csv file or as a graphic (such as a jpeg). Background subtraction can also be accomplished if desired using the “Background” and “Background Options” buttons.

When you are finished

1. Run solvent through the solvent lines for ~3 minutes, once with the injection valve in the LOAD position and once with the injection valve in the INJECT position. This ensures that none of your sample is carried over into the data that the next user collects and also helps prevent the solvent lines from becoming clogged.
2. Click on the Instrument tab and turn off the isocratic pump, or press the play/pause button on the front of the pump.
3. Click the red STANDBY button in the instrument control software to turn off the mass spectrometer voltages and gas flows.
4. Turn off the blue pump on the bench behind the computer.
5. Close the software and log out.
6. End your kiosk session in iLab.

Common Issues and Solutions

Connection error when opening CheMS Express

Solution: Close the instrument software, unplug the USB from the front of the Advion, and plug the USB back in. Then reopen the CheMS Express software.

No solvent flowing through ESI source (no signal)

You can verify whether solvent is flowing by unscrewing the fitting on top of the ESI ion source and dabbing the end of the tubing with a Kimwipe while the solvent pump is on. If the wipe gets wet, solvent is flowing. If not, solvent is not flowing.

Solution 1: Ensure that the solvent bottle has enough solvent for your experiment.

Solution 2: Remove all air bubbles from the clear solvent line by turning the black purge valve counterclockwise and sucking them out using a plastic syringe. Then close the purge valve and turn on the pump to verify that solvent is flowing again.

Solution 3: The solvent lines or ESI ion source may be plugged. Most likely the yellow sample loop is plugged and needs to be purged. You can try increasing the solvent flow rate while the injection valve is in the INJECT position to clear the clog. Unplug the solvent line from the ESI ion source prior to doing this so that the ESI source does not also get clogged. Contact RIC staff if you are unsure how to do this.

High background noise

Solution 1: Run a blank sample using the solvent that your sample is dissolved in to verify that background ions are not from your sample. If the solvent blank background is still high, the solvent lines may need to be cleaned (or your solvent is contaminated). Turn on the solvent pump and run solvent through the lines for several minutes with the injection valve in the LOAD and INJECT positions. Then acquire another blank spectrum to see if the background decreased to an acceptable level.

Solution 2: If the mass spectrometer inlet (which can be viewed through the window in the ESI ion source) is dirty, contact RIC staff. The inlet can be replaced with a clean one that has been sonicated for several minutes in solvent (water/acetonitrile/methanol/formic acid).

Solvent leaking out the injection valve

The injection valve needs to be rebuilt. Please contact RIC staff.

Tune

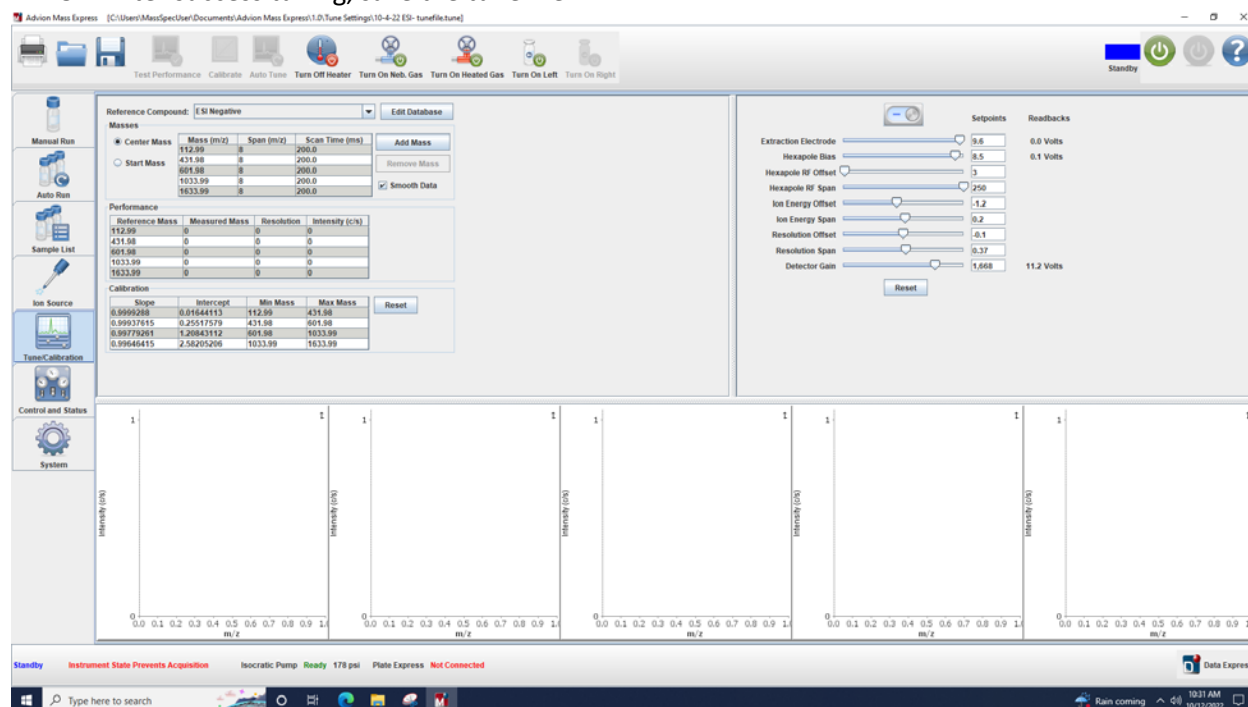
The instrument need to be tuned every one or two month to make sure the ionization condition is optimized.

The tuning solution are as following:

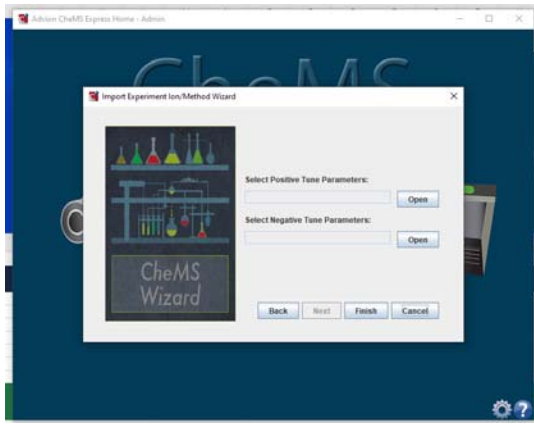
- ESI tuning mix, Agilent technologies, Part No: G2421-60001
- APCI/APPI tuning mix, Agilent technologies, Part No: G2432A

To start tuning,

1. Add tuning mix solution to the tuning mix vial, which is located at the left of the ion source.
2. Disconnect the sample line on top of the ESI ion source and connect the calibration line.
3. Click on “CheMS Mass Express” on Desktop, and choose the “Tune/calibration” tab from the left panel.
4. Click the green button (top right) to switch off standby and turn the voltages and gas flows on.
5. Click the “turn on left” to switch on the calibration solution.
6. Make sure you are selecting same polarity
7. Click “Auto tune”
8. After success tuning, save the tune file.



9. Open “Advion CheMS Express on Desktop and log in with Admin account
10. Click the gear at the lower right and choose import wizard, then choose Tune parameters, then the ion source.
11. Select the corresponding tune file for each polarity, and finish.



12.