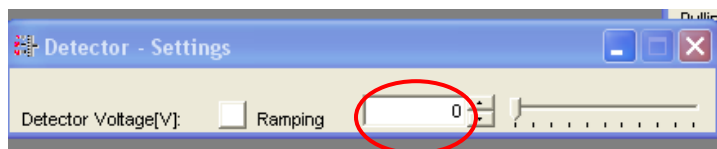


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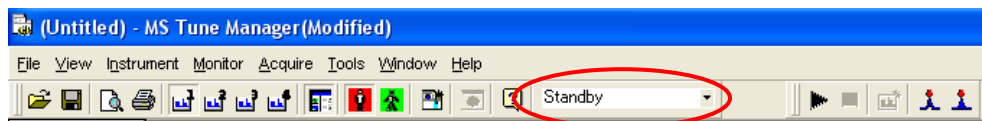
Controlled Substances Instructions for the DART-TOF with MassCenter Software



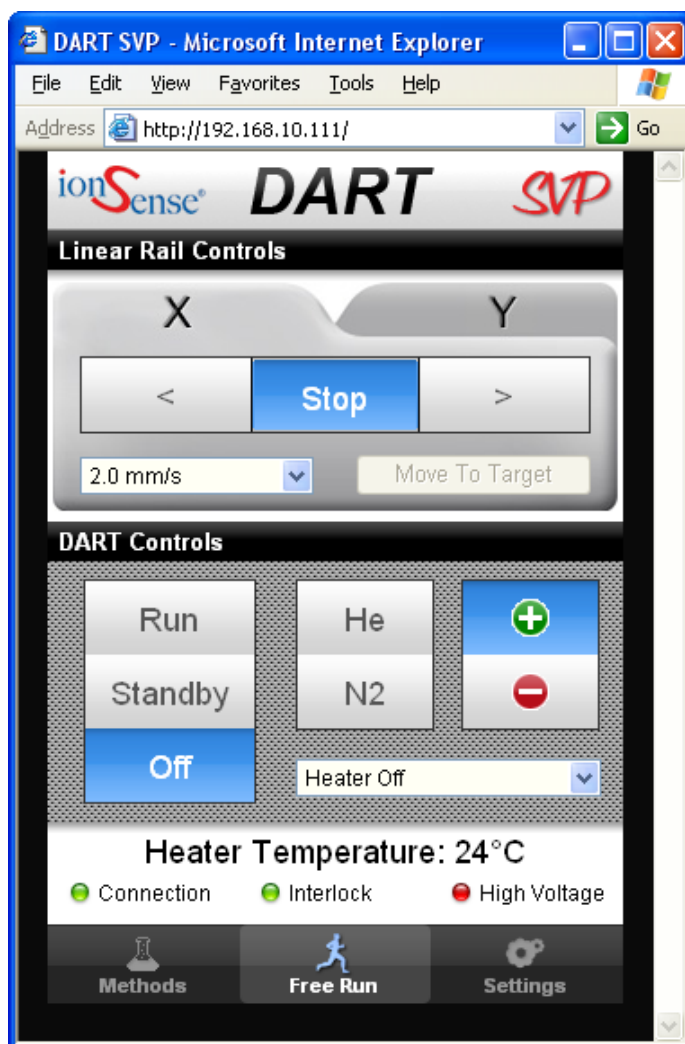
1. In MS Tune Manager, under the File menu, load the DART_POS_O1_20V tune file. Once loaded, make sure the voltage setting for the detector is not zero (~ 1900V).



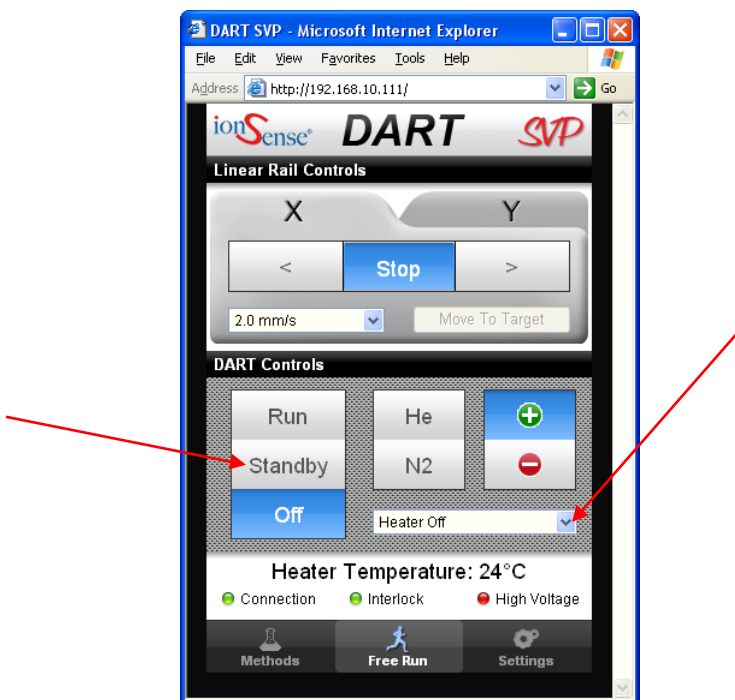
2. Set the AccuTOF to “Operate”. Select the down arrow (next to “Standby” below) and scroll to select “Operate”



3. The DART Control window will either be open or minimized at the bottom of the screen. If it is minimized, open it.



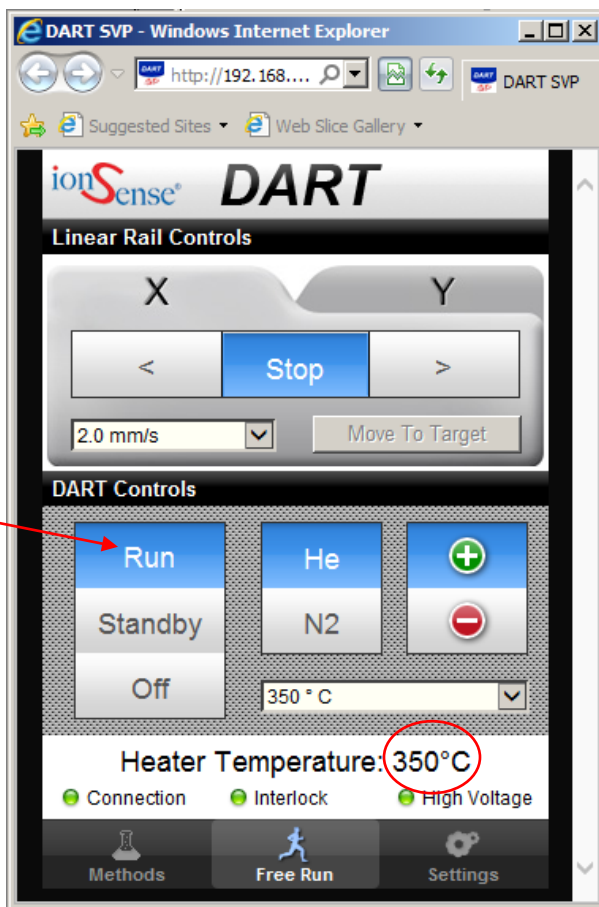
- In the DART Control window, click on “Standby” and then click on the down arrow for the heater control.



- Choose the appropriate DART gas temperature for the type of analysis being performed (typically 350°C for screening and pharmaceuticals).

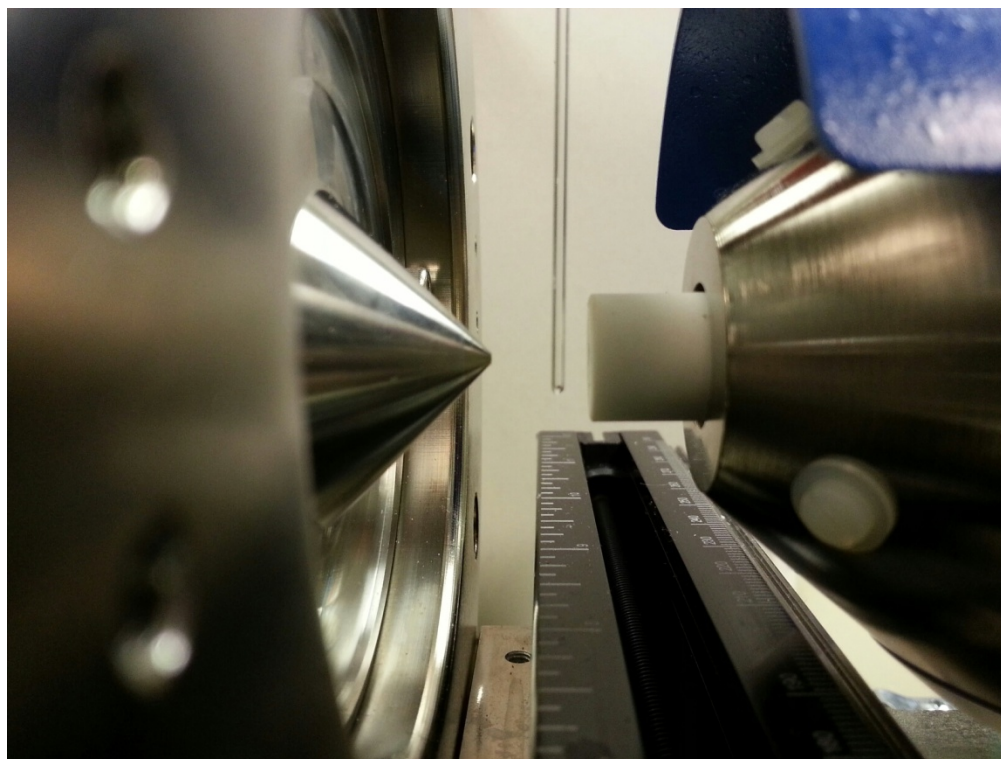


6. Allow DART temperature to rise to the setpoint then click on “Run”.

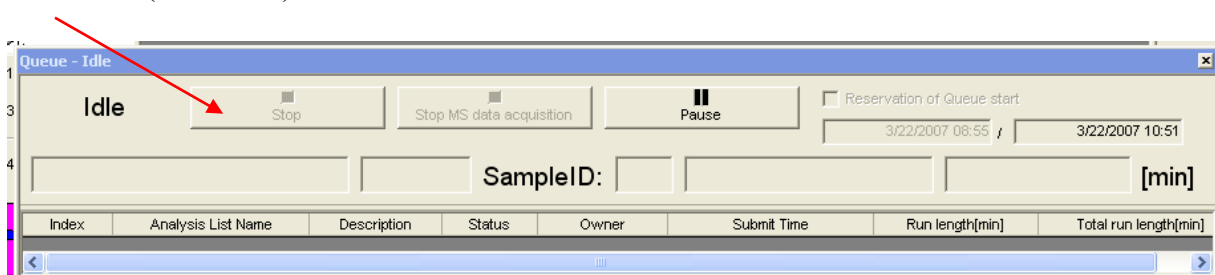


7. In MS Tune Manager, select “Acquire” on the menu bar and “Start Acquisition”, the Acquisition Wizard screen will appear.
 - a. Select “next” and enter the acquisition data (file name) and your internal sample ID information; check to make sure today’s date is in the acquisition data folder box.
 - b. Select “next” and check “use existing MS acquisition method”. This should be “DART_POS_SWITCH_O2 (this method utilizes function switching, changing the orifice 1 voltage every 0.25sec). You can also set the time allowed for the run in this screen (this is dependent on how many samples you want to run).
 - c. Click finish.
 - d. Click on Start Run, then wait ~0.2 min before the next step (sampling the PEG).

8. Run PEG 600, drift compensation/positive control mix and samples.
- Dip the closed end of a melting point tube in the PEG 600 solution and wand (generally 1-3 times) through the sample gap (start with the tube low in the sample gap and draw it up through the gas stream for best results). Stay closer to the ceramic end cap, but be cautious not to touch it with the tube. Remember that after you have wanded the melting point tube through the gas stream, it will be hot!

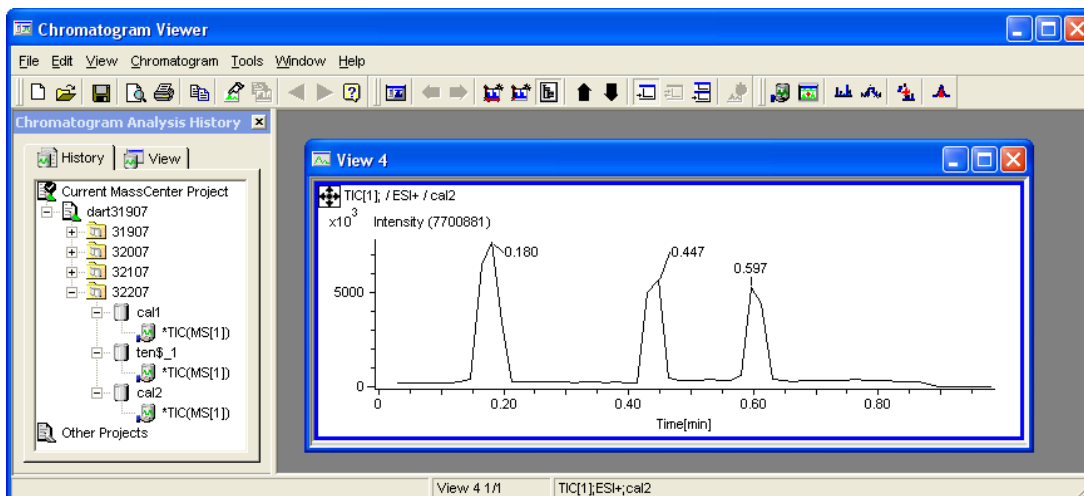


- Similarly, using clean melting point tubes, run the drift compensation/positive control mix (methamphetamine, cocaine and nefazodone) and your sample(s).
- After you are finished collecting data, turn the DART control to “OFF”. Stop acquiring data by clicking “Stop” in the Queue box in MassCenterMain in the lower left portion of the screen (see below).



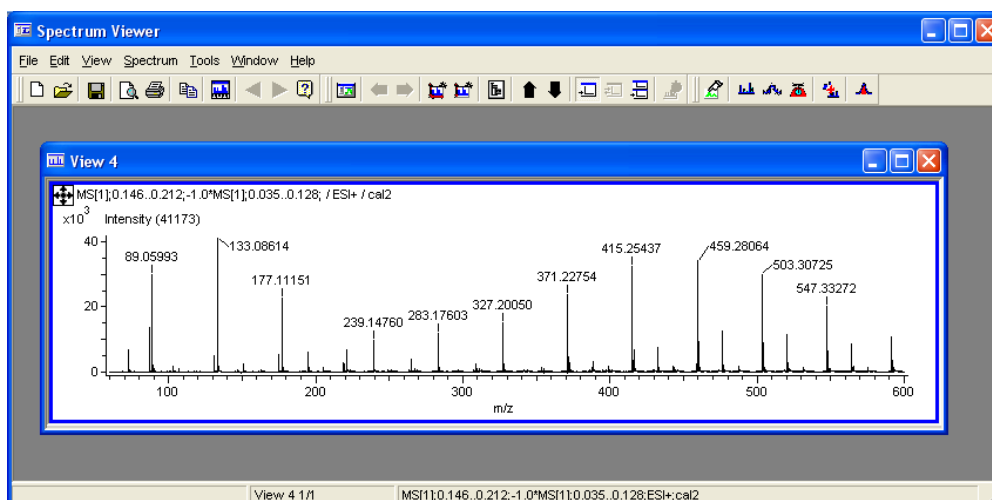
- In the MS Tune Manager window, load the “Overnight” tune file. This will return the MCP detector voltage setting to zero. Then, place the AccuTOF back in “Standby” mode.

9. Open the spectrum viewer and chromatogram viewer windows (in Mass Center Main, under the Tools menu, if they are not already open on the tool bar).
10. Open data file - in the chromatogram viewer window, under the history tab (box on the left), left click on the data file name, then right click and select open (simply double clicking on it will not work!).



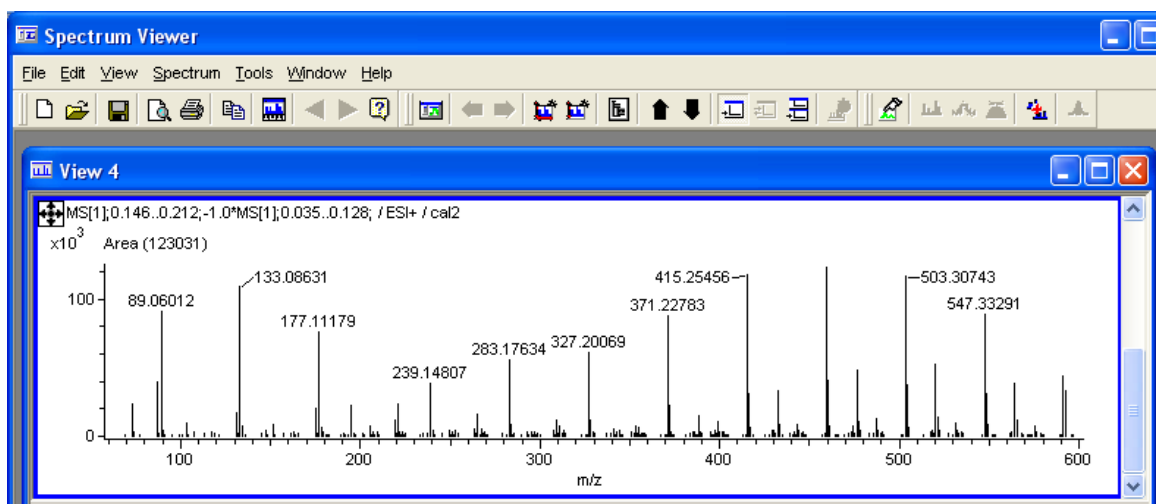
11. Performing the mass calibration:

- a. Scroll to TIC[2] in the chromatogram viewer window (this is the data for orifice 1 voltage = 30 V).
- b. Zoom in on the PEG peaks and take a background subtracted spectrum
 - i. Shift and right-click drag across the peak, then
 - ii. Control and right-click drag across a background area
 - iii. The software will then “draw” the background subtracted, averaged spectrum in the spectrum viewer window (if it doesn’t work properly, try it again!).



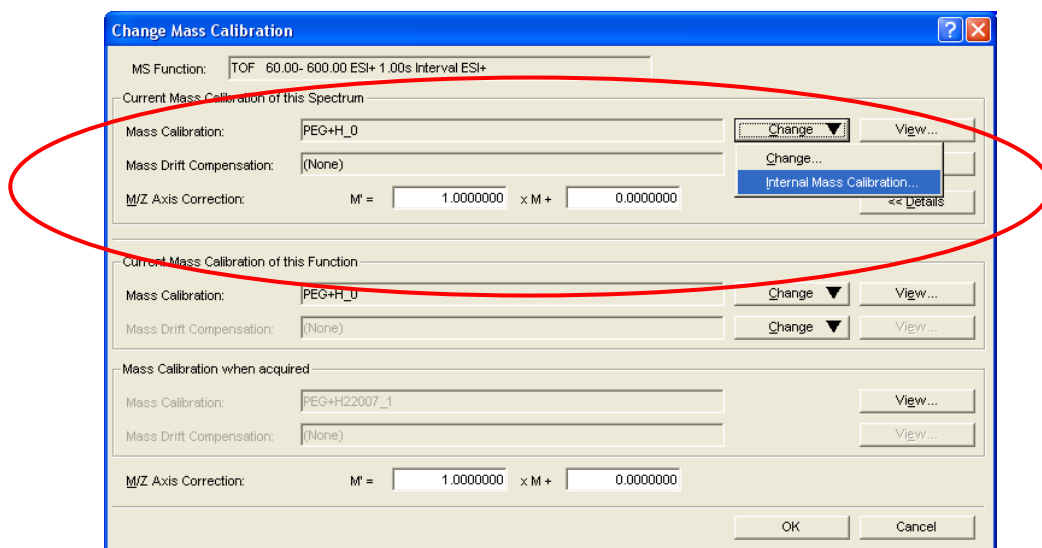
c. Centroiding the spectrum

- i. On the menu bar, select Spectrum, Make Centroid Spectrum (or press Alt, s, m) and click on Apply

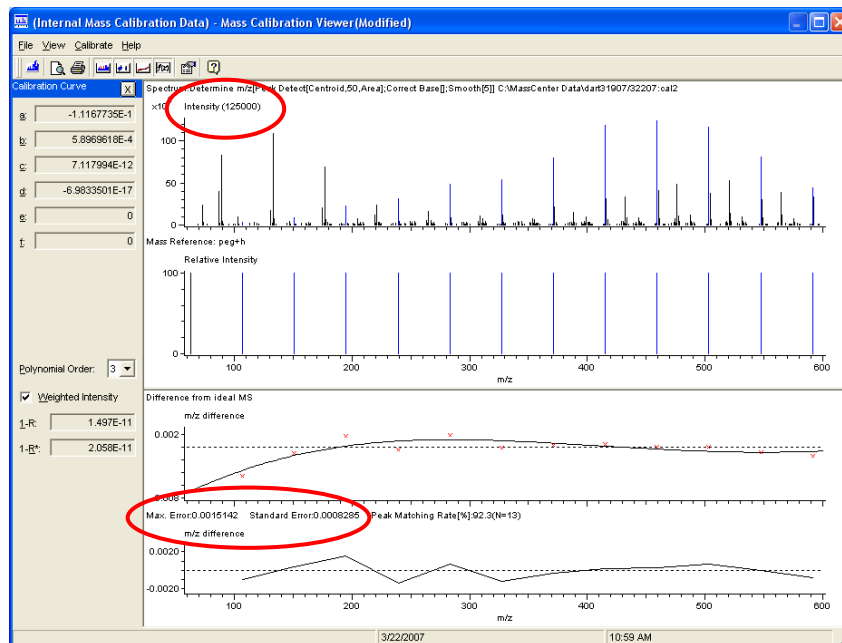


Centroided PEG 600 spectrum

- ii. Close centroid window.
- d. Set mass calibration
 - i. On the menu bar, select Tools, Change Mass Calibration (or press Alt, t, m)
 - ii. Under “Current Mass Calibration of this Spectrum” (Mass Calibration) select Change, Internal Mass Calibration...



- iii. The Internal Mass Calibration Wizard will appear. Click finish.
- iv. This will bring up the Mass Calibration Viewer. Look at the intensity of your spectrum, intensities above 15000 tend to give good mass calibration data.
- v. Max. error should be less than 0.003 and standard error should be less than 0.001



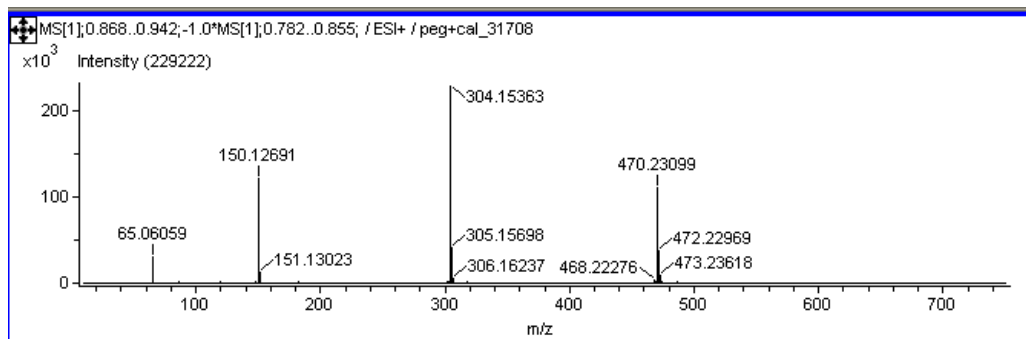
- vi. There are a couple of things that can be done to improve the mass calibration, if necessary.
 1. Change polynomial order to 4. (Use only 3 or 4)

and / or
 2. Remove outlying data points
 - a. Zoom in the outlying point, left click to select item (turning it red), then right click and select “remove assignment” (no more than two non-adjacent points should be removed)
- vii. To save the mass calibration, on the menu bar select File, Save As (or press Alt, f, a) and scroll down to select temp_PEG from the file menu. Click “Save”. Yes, you do want to replace the existing file!
- viii. On the menu bar, select File, Exit then Return (or press Alt, f, r) to return to the previous window.
- ix. Under “Current Mass Calibration of the Function” (Mass Calibration) select Change, then Change..., and select temp_PEG from the menu. Click OK. This sets your mass

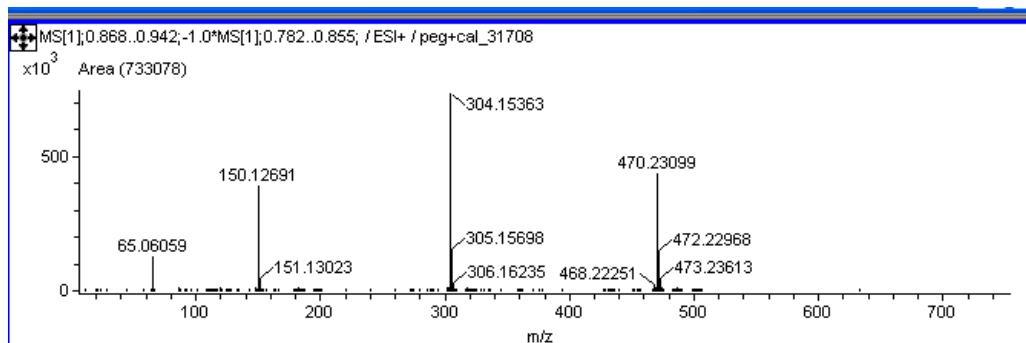
calibration for all of the peaks in this function only. This will need to be done for each function (orifice voltage). Click OK to return to the spectrum window.

12. Performing the mass drift compensation

- a. Zoom in on the drift compensation/positive control mix (methamphetamine, cocaine and nefazodone) peaks in TIC[2] and obtain a background subtracted spectrum (follow the same process as in section 11b.)

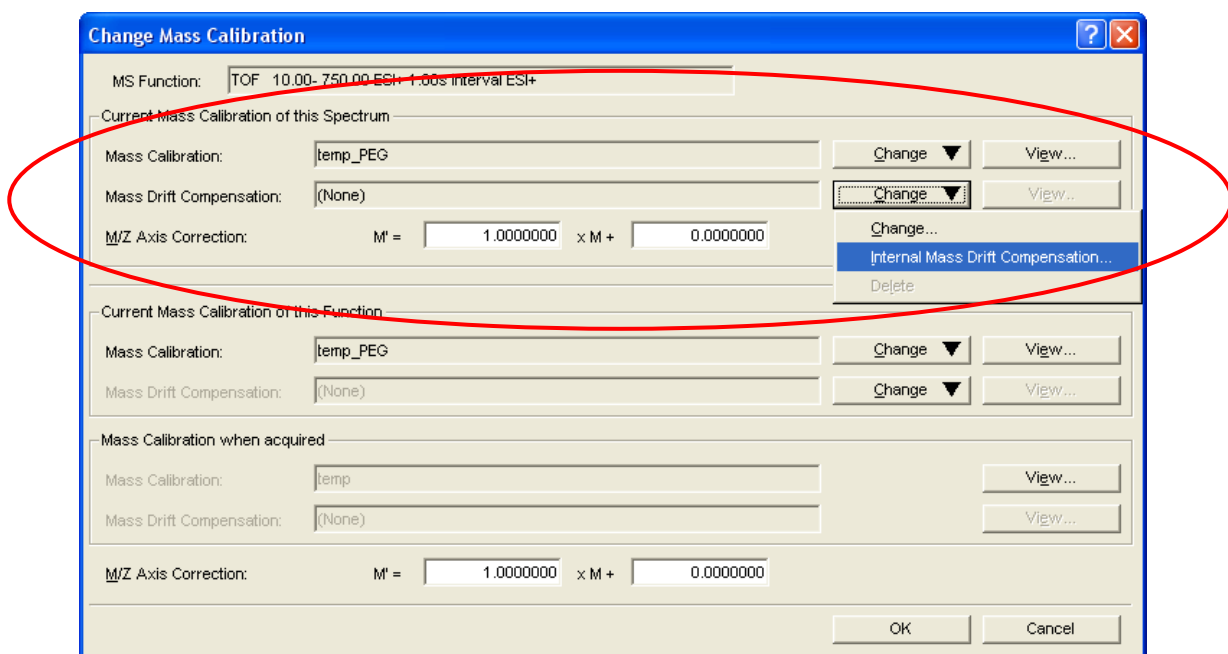


- b. Centroid the spectrum (under the spectrum menu) – or Alt, s, m (see section 11c.)

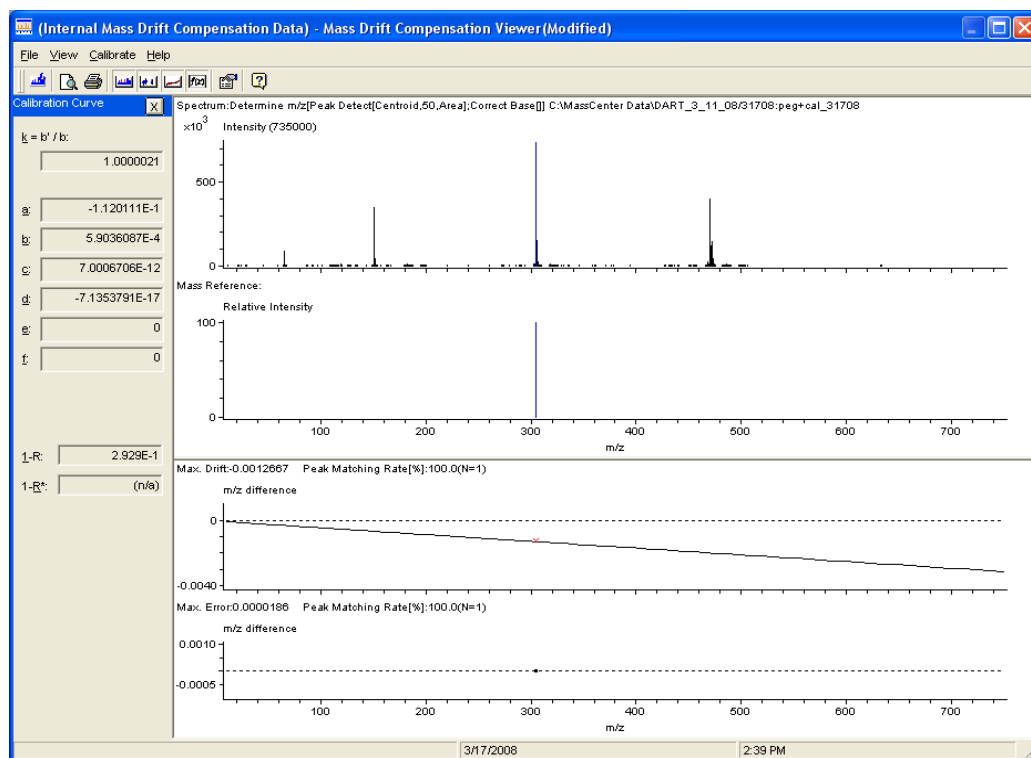


c. Set mass drift compensation

- i. On the menu bar, select Tools, Change Mass Calibration (or press Alt, t, m)
- ii. Under “Current Mass Calibration of this Spectrum” (Mass Drift Compensation) select Change, Internal Mass Drift Compensation...



- iii. The Internal Mass Drift Compensation Wizard will appear. The wizard is set up to lock the protonated cocaine molecule to its calculated mass at 304.1549 Da. Click finish.



- iv. This will bring up the Mass Drift Compensation Viewer.
- v. To save the mass drift compensation, on the menu bar select File, Save As (or type Alt, f, a) and scroll down to select temp_drift from the file menu. Click "Save". Yes, you do want to replace the existing file!

- vi. On the menu bar, select File, Exit then Return (or type Alt, f, r) to return to the previous window.
 - vii. Under “Current Mass Calibration of the Function” (Mass Drift Compensation) select Change, then Change..., and select temp_drift from the menu. Click OK. This sets your mass drift compensation for all of the peaks in this function only. This will need to be done for each function (orifice voltage). Click OK to return to the spectrum window.
- d. Check the values of the methamphetamine and nefazodone positive controls; they need to be within $\pm 5\text{mDa}$ of the calculated protonated molecules at 150.1283 Da and 470.2323 Da, respectively. If they are not within the accepted range, redo the mass calibration using another PEG600 peak. The mass drift compensation MUST be redone if the PEG600 calibration is redone!
 - e. Print out the post-calibration/drift compensation spectrum (from the spectrum viewer) showing the cocaine, methamphetamine and nefazodone peaks and include in your case file, making sure to indicate that you’ve determined the masses to be within the $\pm 5\text{mDa}$ criteria.

13. Data processing

- a. Scroll to TIC[2] (this is the data for orifice 1 voltage = 30 V)
- b. Obtain a background subtracted spectrum for a sample peak (follow the same process as in section 11b.)
- c. Centroid the spectrum (under the spectrum menu) – or Alt, s, m (see section 11c.)
- d. Mass calibration and mass drift compensation have already been set for this function in the previous steps.
- e. To obtain spectra from other functions, scroll to the appropriate function and repeat steps 12b and 12c. Use the following steps to apply the mass calibration and mass drift compensation to the additional functions.
 - i. Set your mass calibration and mass drift compensation for the spectrum- Tools, Change Mass Calibration (Alt, t, m)
 1. Under “Current Mass Calibration of this Spectrum” (Mass Calibration) select Change, then Change..., and select temp_PEG from the menu. Click OK. This sets your mass calibration for this spectrum only. You will then need to change the mass calibration for the rest of the spectra in this function.
 2. Under “Current Mass Calibration of this Spectrum” (Mass Drift Compensation) select Change, then Change..., and select temp_drift from the menu. Click OK. This sets your mass drift compensation for this spectrum only. You will then need to change the mass drift compensation for the rest of the spectra in this function.

- ii. Set your mass calibration and mass drift compensation for this function – with the Change Mass Calibration screen still open,
 1. Under “Current Mass Calibration of the Function” (Mass Calibration) select Change, then Change..., and select temp_PEG from the menu. Click OK. This sets your mass calibration for all of the peaks in this function.
 2. Under “Current Mass Calibration of the Function” (Mass Drift Compensation) select Change, then Change..., and select temp_drift from the menu. Click OK. This sets your mass drift compensation for all of the peaks in this function.
- f. Saving your data – File, Save As, JEOL_DX (or type Alt, F, A, J). Be sure to choose the proper location for saving your data: C:\MassCenterData\project file\folder\datafile. The project file (*.7pj) should be the latest project (by date) and the folder (*.7fl) should be the current date.
- g. “Library searching”
 - i. Open “Mass Mountaineer” (it should be open on the menu bar, if not select the shortcut on the desktop)

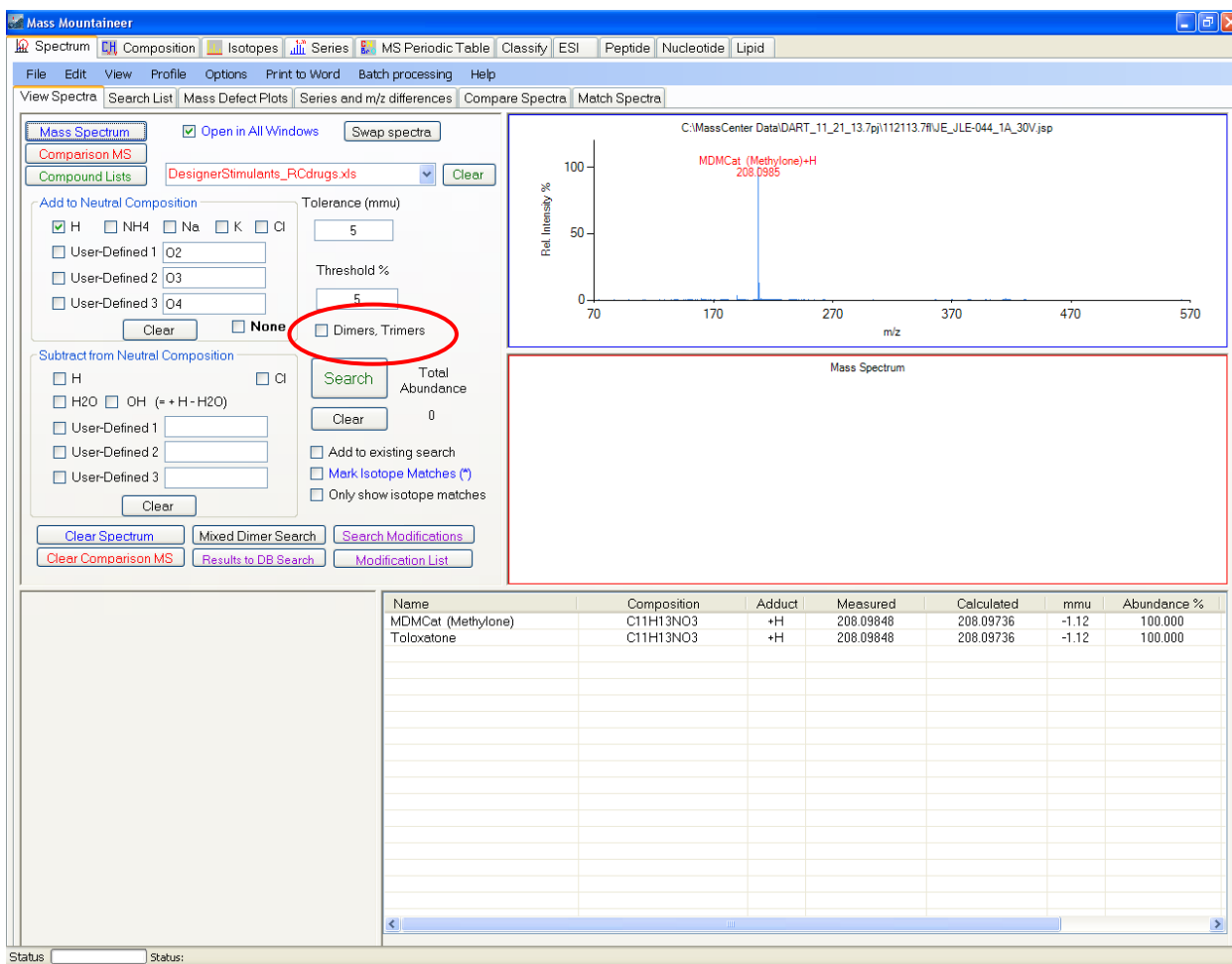
Name	Composition	Adduct	Measured	Calculated	mmu	Abundance %
MDMCat (Methylone)	C11H13NO3	+H	208.09848	208.09736	-1.12	100.000
Toloxatone	C11H13NO3	+H	208.09848	208.09736	-1.12	100.000

- ii. Open your MS file by clicking on the Mass Spectrum button and follow the path to your datafile (C:\MassCenterData\project file\folder\datafile)

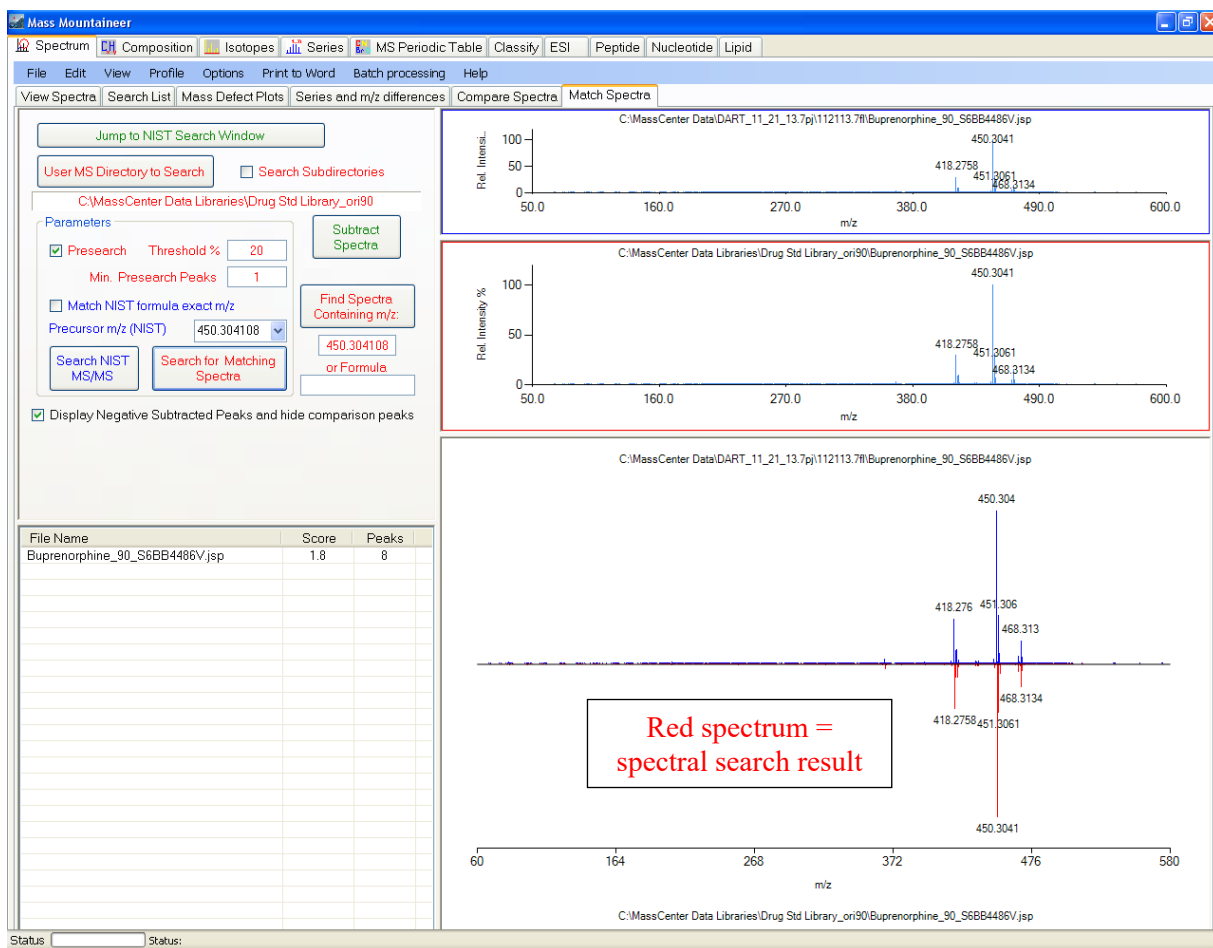
- iii. Load your search list(s) by clicking on the Compound Lists button and navigating to the Libraries (C:\MassCenter Data Libraries\library name.xls). Multiple search lists can be open at one time. Click on the down arrow to see what lists are currently open. Removing a search list can only be done by clearing all the lists and re-building with the list(s) you want/need.
- iv. Set your threshold % and mass tolerance (threshold 5, mass tolerance 5 is a good place to start)
- v. Make sure the charge carrier (in the “Add to Neutral Composition” box) is selected, typically “H”, by checking the box. User defined adducts can be used here. There may be special circumstances when you’d want to select another choice (for example, if you’ve used ammonia to “derivatize” your sample).

The screenshot shows the Mass Mountaineer software interface. The 'Add to Neutral Composition' section has the 'H' checkbox checked and circled in red. The 'DesignerStimulants_RCdrugs.xls' search list is selected. The 'Mass Spectrum' plot shows a single peak at m/z 208.0985 labeled 'MDMCat (Methylon 208.0985)'. The 'Tolerance (mmu)' is set to 5 and 'Threshold %' is set to 5. The 'Search' button is highlighted in green.

- vi. Click on Search. Checking the “Dimers, Trimers” box will allow searching for these adducts. Search results will be displayed in list form at the bottom of the screen and peaks in the spectrum will be labeled with search hits (if the compound Name is too long, the label might not be applied). Clicking the “Calculated” tab in the search results table will order the results by increasing mass. Print your data using “File/Print Now” or “ctrl-P” on the keyboard.



- vii. To compare data against the spectral libraries, load your spectrum by clicking on the Mass Spectrum button, then click on the Match Spectra tab.
- viii. Click on the User MS Directory to Search button and navigate to the MassCenter Data Libraries folder and select the spectral library to search (prep libraries for most pharmaceutical preparations and std libraries for primary standards spectra). Be sure to choose the appropriate orifice 1 voltage for the data spectrum you are searching.
- ix. Click on the Search for Matching Spectra button. The status bar at the bottom left will display the search progress.
- x. The resulting search will show your data spectrum on the top, the comparison spectrum from the search result and a mirror display with your data at the top (in blue) and the comparison in red. Tabular results are displayed at the bottom left. Clicking on subsequent entries in the search list will display the spectrum in the mirror and comparison windows. Print data using “File/Print Now” or “ctrl-P” on the keyboard.



xi. Repeat as necessary for other orifice voltages.

14. When you have finished using the DART, ensure the gas temperature is below $\sim 120^{\circ}\text{C}$ and then click on the "Off" button to turn the unit off.