

Tuning and Optimizing Your ETD Source for the Best ETD Performance

Supplemental information can be found at:

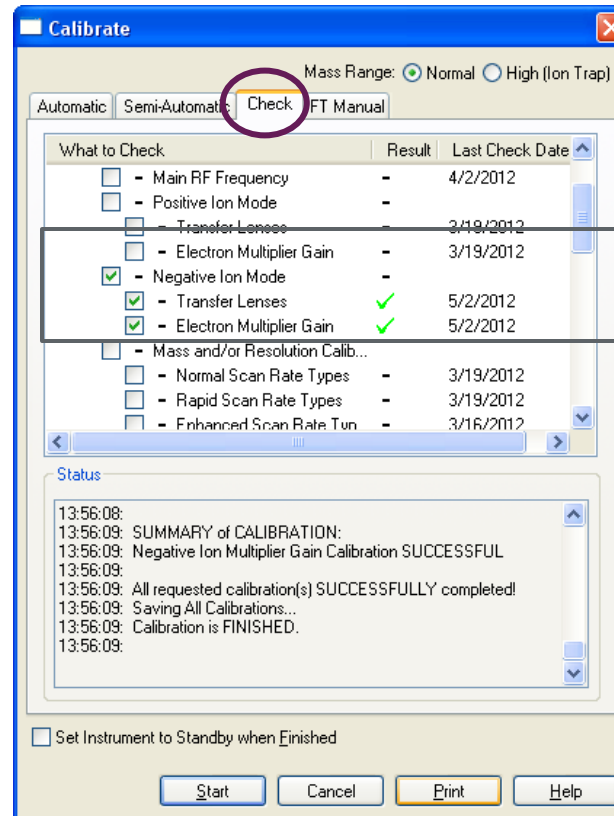
http://sjsupport.thermofinnigan.com/TechPubs/manuals/ETD_Start.pdf

Reagent ion signal requirements for optimum ETD performance

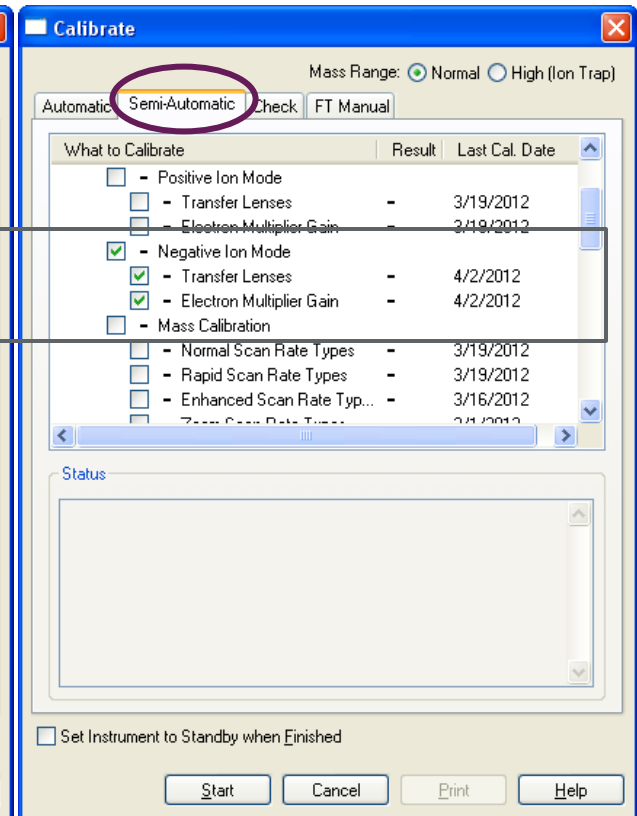
- Turn on ETD source and allow 30 minutes for reagent ion signal to stabilize.
- Minimum intensity required is at least $5e5$ in profile mode for optimal LCMS timescale experiments. Anticipated reagent signal for a well tuned source is approximately $1e7$ in profile mode.
- How to optimize ETD reagent signal
 - Check multiplier gain calibration routine in both positive and negative polarity. If necessary, recalibrate.
 - Check reagent ion selection calibration. If necessary recalibrate. Also, insure that Reagent ion isolation is turned on.
 - Check the transfer mode calibration in both polarities. If necessary, recalibrate.
 - Tune the ETD ion optics
 - Optimize the Chemical Ionization (CI) gas pressure
 - Change or clean the ion volume
 - Change filament
 - Change the reagent vial
- Instructions for the above steps in **purple** are detailed on the following slides

Multiplier and the transfer lenses must be calibrated in negative mode

- Negative mode multiplier calibration is necessary for AGC regulation of the anion in negative mode.
- First check the negative mode multiplier calibration. If it fails, continue to calibration.
 - Use negative mode calibration mixture.
- The same holds true for the transfer lenses



Check Tab



Calibrate Tab

Turn On the ETD Source

The screenshot displays the Thermo Tune Plus software interface. The main window shows a mass spectrum plot with a peak at m/z 202.0833. The Reagent Ion Source dialog box is open, showing the following settings:

Parameter	Set Value	Actual Value
Reagent Ion Source On	<input checked="" type="checkbox"/>	On
Filament On	<input checked="" type="checkbox"/>	On
Emission Current (uA)	50.00	51.45
Electron Energy (V)	-70.00	
CI Gas Pressure (psi)	9.60	9.63
Source Temp (°C)	160	160.09
Vial 1 Temp (°C)	90.00	90.12
Restrictor Temp (°C)	160.00	160.11
Transfer Line Temp (°C)	160.00	159.92
Reagent Ion from Vial 1:	Fluoranthene (m/z: 202.00)	
View Reagent Ion Spectra	<input checked="" type="checkbox"/>	

The status panel on the right shows various system parameters, including NSI Source, Vacuum, FT Vacuum, and Turbo Pump status.

On TUNE page

- Load a good Tune file for ETD.
- Access the ETD source GUI.
- Turn on reagent ion source, (takes some time to get vial to temperature).
- Turn on filament.
- Click view reagent ion spectra (202 m/z).
- The expected 202 signal intensity is $>5e5$ in profile mode for LCMS experiment.
- Tuning and optimization of ETD source is necessary when 202 level is lower than expected.

Be sure vial temp is set to 90

Tuning ETD ion optics

Click the Tune icon to open the tune tab.

View the plot of tuning procedure here.

The screenshot displays the Thermo Tune Plus software interface. At the top, the title bar reads "Thermo Tune Plus - C:\Xcalibur\data\Sucharita\NMR_Shah\NSH.LTQTune". The main window features a menu bar (File, View, Control, Settings, Display, Setup, Diagnostics, Help) and a toolbar with various icons. A central schematic diagram of the Orbitrap Velos Pro ion trap is shown with the text "Scanning...".

On the left, a mass spectrum plot shows "Relative Intensity" on the y-axis (0 to 100) and "m/z" on the x-axis (195 to 225). A prominent peak is labeled at m/z 202.0833. Other labeled peaks include 194.0833, 201.2500, 203.0833, 204.0000, 215.1667, 216.0833, and 219.0833. The plot title is "#24706 IT: 0.365 ST: 0.15 uS: 1 CS: 1 AMW: 203.09 NL: 57.0E6 F: ITMS - p Cl Full ms [100.00-1000.00]".

In the center, a "Tune" dialog box is open, showing "Optimization Items" and "Status". The "Optimization Items" list includes: FT Reagent Ion Transfer Multipole Offset, FT Reagent Ion Transfer Multipole Amplitude, FT Reagent Ion Gate Lens, FT Reagent Ion Transfer Multipole Amplitude, FT HCD Backlens Offset, FT HCD Offset, FT HCD Amplitude, FT HCD Entrance Lens Offset, FT Trap Lens Offset, FT C-Trap Offset, and FT Gate Lens Offset. The "Status" section shows a log of optimization steps, including "Adjusting LPT Center Section" and "Adjusting Center Lens".

On the right, a plot titled "Adjusting Center Lens" shows "Lens Potential (V)" on the x-axis (0 to 20) and "Relative Intensity" on the y-axis (0 to 800,000). The plot shows a sharp peak at approximately 6V, with a red line representing the current state and a blue line representing the target or previous state.

At the bottom right, a "User" panel displays a list of system parameters and their values, such as "NSI Source", "Vacuum", "Turbo Pump", and "FT Turbo Pump 1".

Tuning ETD ion optics (continued)

From the automatic tune tab, click Start. Automatic tuning will commence.

The screenshot shows the Thermo Tune Plus software interface. The main window displays a mass spectrum with a peak at 202.0833 m/z. A dialog box titled 'Automatic' is open, showing a list of parameters to be tuned, including Reagent Ion Lens 1, Reagent Ion Gate Lens, Reagent Ion Lens 2, Reagent Ion Lens 3, Back Lens Offset, and various FT (Fast Transfer) offsets. The 'Start' button is highlighted. The status window on the right shows the following parameters:

Label	Value
NSI Source	
✓ Spray Voltage (kV)	1.50
✓ Spray Current (µA)	0.21
✓ Sheath Gas Flow Rate	0.00
✓ Aux Gas Flow Rate	0.01
✓ Sweep Gas Flow Rate	0.00
✓ Capillary Temp OK	Yes
✓ Capillary Temp (°C)	200.05
Vacuum	
✓ Vacuum OK	Yes
✓ Ion Gauge Pressure OK	Yes
✓ Ion Gauge	On
✓ Ion Gauge (E-5 Torr)	1.75
✓ Convection Pressure OK	Yes
✓ Convection Gauge (Torr)	1.19
FT Vacuum	
✓ FT Penning Pressure OK	Yes
✓ FT Penning Gauge (E-10 ...)	0.67
✓ FT Pirani Gauge 1 (Torr)	1.07
✓ FT Pirani Gauge 2 (Torr)	0.00
Turbo Pump	
Status:	Running
Life (hours):	8998
Speed (Hz):	799
Power (Watts):	71
Temperature (°C):	44
FT Turbo Pump 1	
Status:	Running
Life (hours):	9515
Speed (Hz):	1500
Power (Watts):	14
FT Turbo Pump 2	
Status:	Running
Life (hours):	9513
Speed (Hz):	1500

- Retune until improvement in signal is less than 20%.
- Tuning of individual lens elements can be accomplished through the Semi-Automatic tab
- **When tuning is finished, save the Tune file (click File/Save As).**
 - **Note: these ETD tune settings are only saved to this file. Be sure to tune with the appropriate source (n-ESI, H-ESI II etc.) that you will be using for your experiment**

CI Gas Pressure Optimization

CI gas pressure optimization needs to be done every time the ion volume or filament is replaced. From the Diagnostics tab check the Reagent CI gas pressure evaluation, then click Start.

After CI gas evaluation finishes, manually enter the optimal CI gas pressure into the reagent source GUI and save the Tune file.

The screenshot displays the Thermo Tune Plus software interface. The main window shows a mass spectrum plot with 'Relative Intensity' on the y-axis (0 to 100) and 'm/z' on the x-axis (195 to 225). A peak is labeled at m/z 203.0833. The 'Diagnostics' window is open, showing a list of procedures. The 'Reagent CI gas pressure evaluation' option is checked. The 'Reagent Ion Source' dialog box is also open, showing various parameters and their actual values.

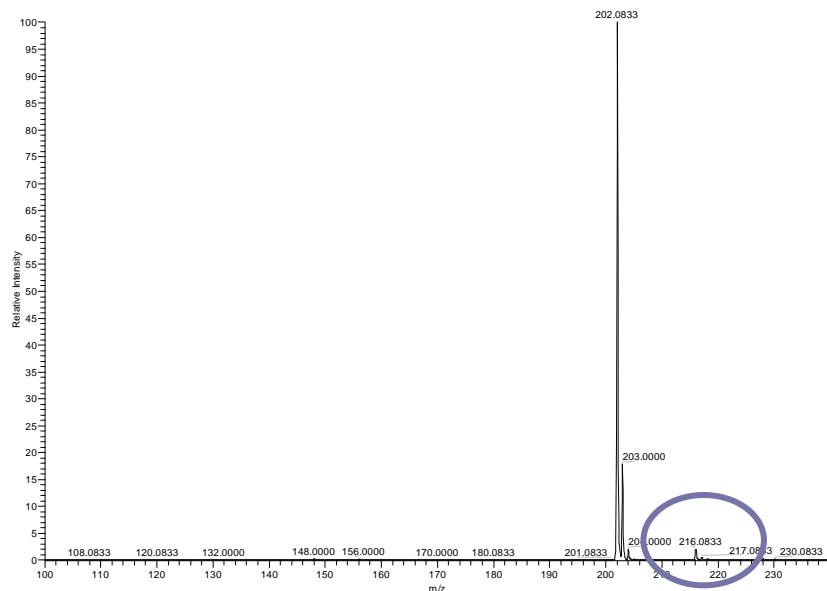
Parameter	Set Value	Actual Value
Reagent Ion Source On	<input checked="" type="checkbox"/>	On
Filament On	<input checked="" type="checkbox"/>	On
Emission Current (uA)	50.00	51.45
Electron Energy (V)	-70.00	
CI Gas Pressure (psi)	9.60	9.63
Source Temp (°C)	160	160.09
Vial 1 Temp (°C)	90.00	90.12
Restrictor Temp (°C)	160.00	160.11
Transfer Line Temp (°C)	160.00	159.92
Reagent Ion from Vial 1	Fluoranthene (m/z: 202.00)	

Buttons in the Reagent Ion Source dialog include: Open Probe Interlock, View Reagent Ion Spectra, Apply, OK, Cancel, Help.

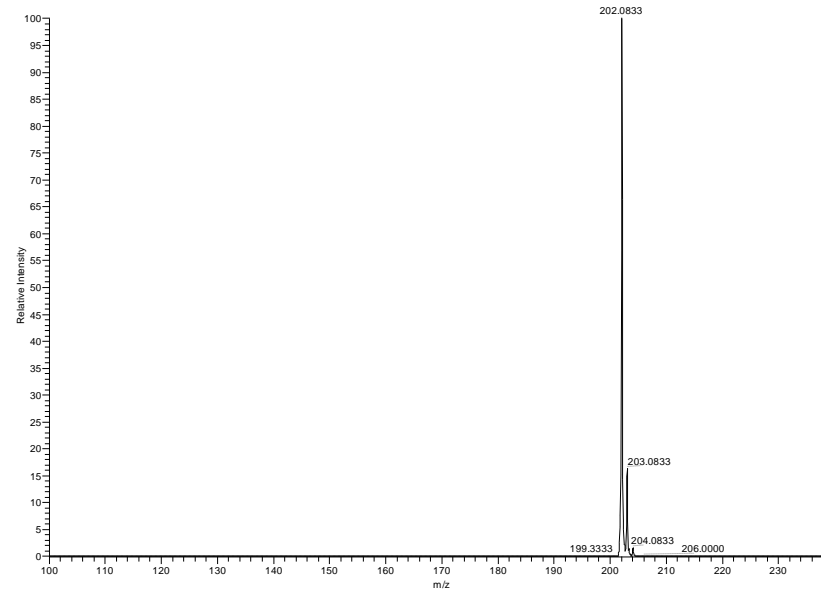
Reagent Ion Isolation should be turned on to eliminate unwanted side reactions

Reagent ion isolation **OFF** → Reagent ion isolation **ON**

#25505 IT: 0.370 ST: 0.15 uS: 1 CS: 1 AMW: 203.09 NL: 5.53E6
F: ITMS - p Cl Full ms [100.00-1000.00]



#25602 IT: 0.636 ST: 0.17 uS: 1 CS: 1 AMW: 203.09 NL: 3.49E6
F: ITMS - p Cl Full ms [100.00-1000.00]



Contaminating anions.

Anion contamination will vary depending on source cleanliness, reagent purity, and Cl gas purity. Shown above is a case of low level contamination.

How to turn on reagent ion isolation

Toggle button to On, then click Set

The screenshot shows the Thermo Tune Plus software interface. A 'Diagnostics' dialog box is open, displaying a list of diagnostic tools and tests. The 'Isolate reagent ion' option is selected, and its toggle button is set to 'On'. The 'Set' button is also highlighted. The background shows a mass spectrum plot with a peak at m/z 202.0833 and a graph titled 'Adjusting Center Lens'.

Diagnostics Dialog Box:

- Tools: Plot readback, Set device, Device calibration, Display settings, Triggers, Mass calibration, System evaluation
- Tests: Readback (FT zero offset, Injection waveforms, Ion gauge check, **Isolate reagent ion**, Main RF loop, Multiplier protect, Normalized coll. energy, Ready out, Reagent ion AGC, RF system, Sheath gas flow protect, Show AGC scan), Testing

Parameter List (Right Panel):

Label	Value
NSI Source	
✓ ISpray Voltage (kV):	1.60
✓ Spray Current (µA):	0.21
✓ Sheath Gas Flow Rate:	-0.09
✓ Aux Gas Flow Rate:	-0.01
✓ Sweep Gas Flow Rate:	-0.01
✓ Capillary Temp OK:	Yes
✓ Capillary Temp (°C):	200.02
Vacuum	
✓ Vacuum OK:	Yes
✓ Ion Gauge Pressure OK:	Yes
✓ Ion Gauge:	0n
✓ Ion Gauge (E-5 Torr):	1.75
✓ Convectron Pressure OK:	Yes
✓ Convectron Gauge (Torr):	1.19
FT Vacuum	
✓ FT Penning Pressure OK:	Yes
✓ FT Penning Gauge (E-10 ...):	0.68
✓ FT Pirani Gauge 1 (Torr):	1.08
✓ FT Pirani Gauge 2 (Torr):	0.00
Turbo Pump	
Status:	Running
Life (hours):	9998
Speed (Hz):	800
Power (Watts):	69
Temperature (°C):	44
FT Turbo Pump 1	
Status:	Running
Life (hours):	9515
Speed (Hz):	1500
Power (Watts):	13
FT Turbo Pump 2	
Status:	Running
Life (hours):	9513
Speed (Hz):	1500

Check reagent ion selection and reagent ion source transfer multipole RF frequency

- No calibration mix is necessary.
- If checks fail, continue to calibration.

