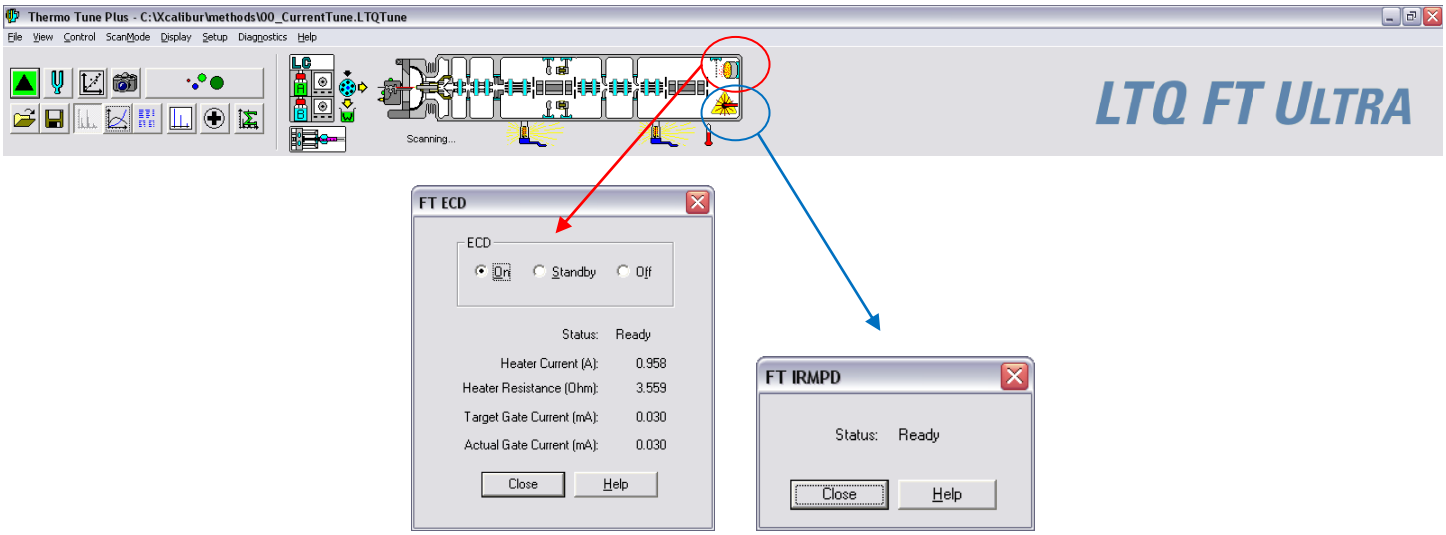
ECD and IRMPD maintenance

Note this document is intended to help guide you through some basic ECD and IRMPD maintenance. For a comprehensive maintenance guide refer to Thermo's manuals and online Help pages. Please let us know if you see any errors or have suggestions to improve this document.

Contents

ECD/IRMPD windows



ON:

When the ECD is in use leave it on. Only put it in standby if it is not in use for an extended period of time. When the ECD is on the symbol will appear bright yellow as you see in the screen shot above.

Standby:

When not in use for extended period of time put ECD in standby (don't turn it off); in standby there is a trickle charge through the cathode.
When turning ECD on out of standby it takes about 1-2hrs before it is ready, the symbol will show a little round timer.
If it returns to standby run the ECD activation.

Off (e.g. when venting the instrument):

When venting the system it is important to turn the ECD off about 1hr before venting to allow the filament to cool down. It is beneficial to vent the instrument with the N2 gas supply on, this way the vacuum is replaced with "dry" N2 gas rather than air.

IRMPD

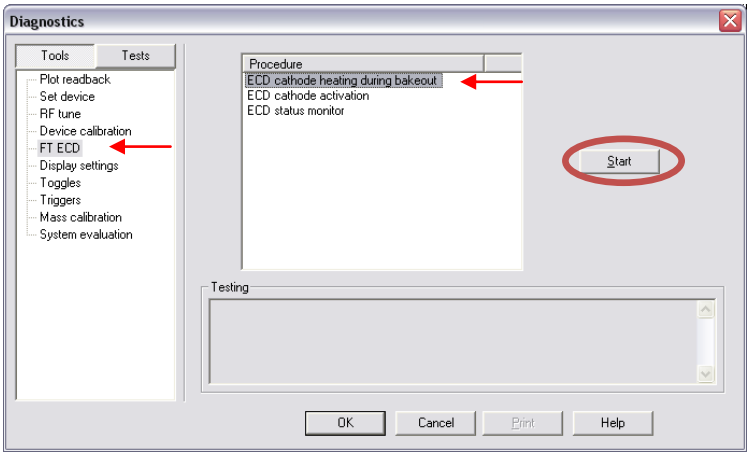
This is just a status window, if the laser is not ready the symbol will appear gray and the Status will read back Not Ready.

ECD Maintenance

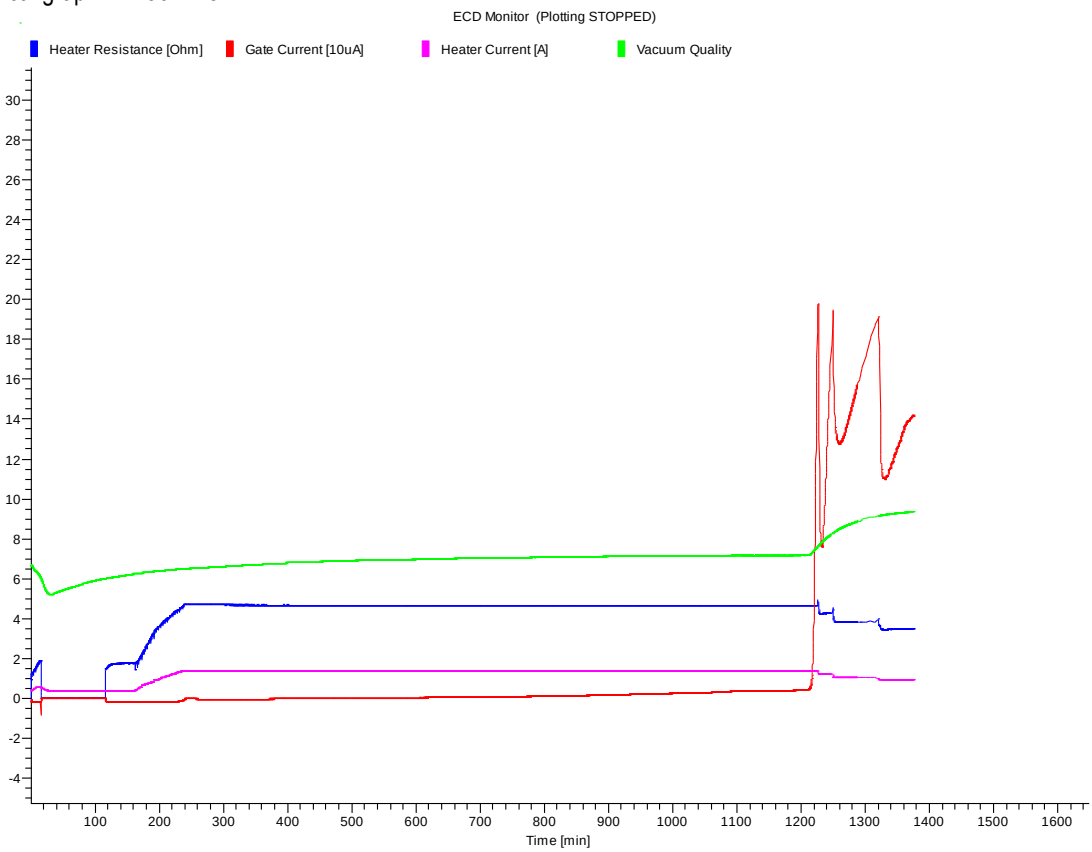
ECD Bakeout

Start the ECD cathode heating during bakeout (Diagnostics/FT ECD/ECD cathode heating during bakeout): hit ***start*** when starting bakeout and stop (note the start button will become the stop button) it when system has cooled down; always activate ECD after bakeout.

Note from the Help Page: During a system bakeout of an LTQ FT Ultra equipped with an ECD unit, it is necessary to keep the ECD cathode temperature at or above the tube temperature to protect the cathode from being poisoned by the bakeout gas products. The ECD cathode heating during bakeout procedure ensures a controlled heating of the ECD cathode. During cathode heating, the gate current and the vacuum quality are monitored. This procedure should be started at each system bakeout. Further details of the system bakeout procedure are discussed in the LTQ FT Ultra Hardware Manual. While this procedure is active, the heater current, heater resistance, vacuum quality, and gate current are displayed in the Graph view.



This is what a typical graph will look like.

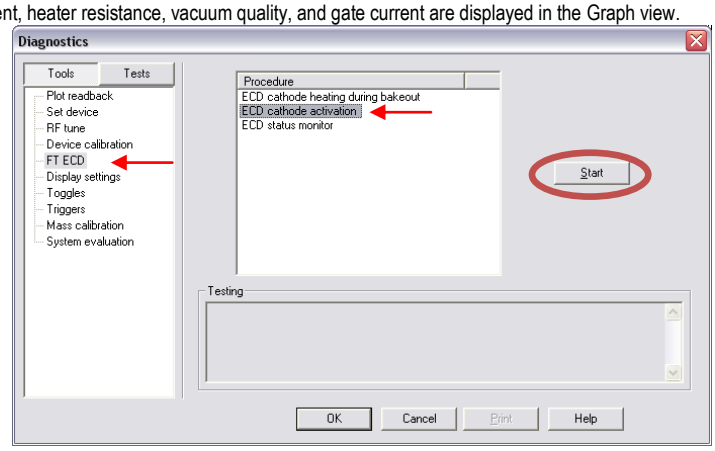


ECD cathode heating during bakeout

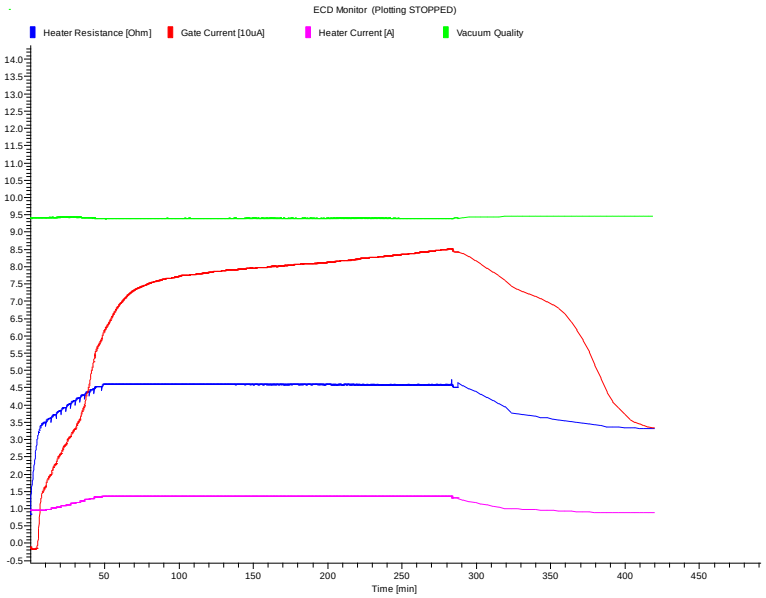
ECD Activation

With ECD turned on and placed *inside* the magnet start ECD cathode activation. The ECD should be activated after every bakeout. Note sometimes the ECD will not stay on after being in standby for an extended period of time. To resolve this issue we run the ECD activation.

Note from the Help Page: The activation of the ECD cathode is necessary if the system was vented. During the activation, the cathode is kept at a very high temperature while the gate current and the vacuum quality are monitored. The activation of the cathode is achieved by converting the barium oxide in the tungsten matrix into free barium on the surface of the cathode. The rate of activation is a function of tube cleanliness, cathode poisoning, time, and temperature. All systems are different so there is no "standard" activation schedule. Cathode electron emission is the best indicator of activation. The activation procedure takes 5 to 6 hours. The activation is automatically followed by a heater current calibration.
Note. The gate current depends on whether the ECD unit is placed inside or outside the magnet. Thus an ECD cathode activation should always be performed when the ECD unit is in the magnet.
While this procedure is active, the heater current, heater resistance, vacuum quality, and gate current are displayed in the Graph view.



This is what a typical graph will look like.

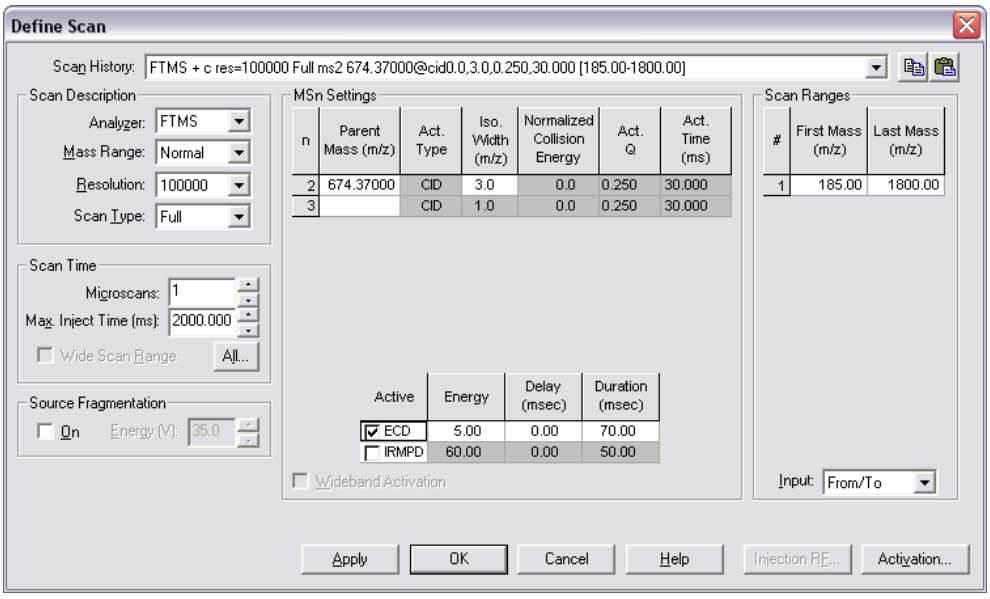


ECD cathode activation

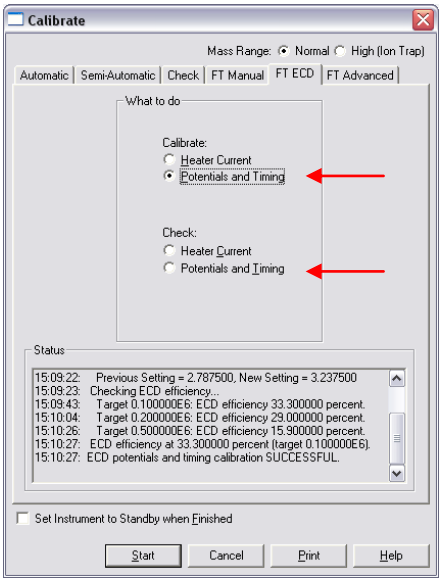
13:47:14: Start FT ECD cathode activation
13:47:15: Gate current at -0.001000 mA, (heater 0.945000 A) ...
13:49:15: Gate current at -0.002000 mA, (heater 0.945000 A) ...
13:51:15: Gate current at -0.001000 mA, (heater 0.945000 A) ...
13:53:15: Gate current at 0.006000 mA, (heater 0.945000 A) ...
13:55:15: Gate current at 0.014000 mA, (heater 0.945000 A) ...
13:57:15: Gate current at 0.016000 mA, (heater 0.945000 A) ...
14:09:15: Gate current at 0.028000 mA, (heater 1.085000 A) ...
14:21:15: Gate current at 0.037000 mA, (heater 1.225000 A) ...
14:30:21: ECD Activation: Starting 4h burn-in period.
14:33:15: Gate current at 0.057000 mA, (heater 1.330000 A) ...
14:45:15: Gate current at 0.068000 mA, (heater 1.365000 A) ...
14:57:15: Gate current at 0.073000 mA, (heater 1.365000 A) ...
15:09:15: Gate current at 0.075000 mA, (heater 1.365000 A) ...
15:21:15: Gate current at 0.077000 mA, (heater 1.365000 A) ...
15:33:15: Gate current at 0.078000 mA, (heater 1.365000 A) ...
15:45:15: Gate current at 0.078000 mA, (heater 1.365000 A) ...
15:57:15: Gate current at 0.079000 mA, (heater 1.365000 A) ...
16:09:16: Gate current at 0.079000 mA, (heater 1.365000 A) ...
16:21:15: Gate current at 0.080000 mA, (heater 1.365000 A) ...
16:33:15: Gate current at 0.080000 mA, (heater 1.365000 A) ...
16:45:15: Gate current at 0.081000 mA, (heater 1.365000 A) ...
16:57:15: Gate current at 0.081000 mA, (heater 1.365000 A) ...
17:09:15: Gate current at 0.081000 mA, (heater 1.365000 A) ...
17:21:15: Gate current at 0.082000 mA, (heater 1.365000 A) ...
17:33:15: Gate current at 0.083000 mA, (heater 1.365000 A) ...
17:45:15: Gate current at 0.083000 mA, (heater 1.365000 A) ...
17:57:15: Gate current at 0.084000 mA, (heater 1.365000 A) ...
18:09:15: Gate current at 0.084000 mA, (heater 1.365000 A) ...
18:21:15: Gate current at 0.085000 mA, (heater 1.365000 A) ...
18:30:27: ECD Activation: Burn-in period ends, start check.
18:30:28: ECD Gate current above target, start regulation.
18:33:15: Gate current at 0.084000 mA, (heater 1.324000 A) ...
18:45:15: Gate current at 0.083000 mA, (heater 1.208000 A) ...
18:57:15: Gate current at 0.080000 mA, (heater 1.103000 A) ...
19:09:15: Gate current at 0.076000 mA, (heater 1.007000 A) ...
19:21:15: Gate current at 0.073000 mA, (heater 0.984000 A) ...
19:33:15: Gate current at 0.071000 mA, (heater 0.962000 A) ...
19:45:15: Gate current at 0.068000 mA, (heater 0.941000 A) ...
19:57:15: Gate current at 0.062000 mA, (heater 0.920000 A) ...
20:09:15: Gate current at 0.051000 mA, (heater 0.899000 A) ...
20:21:15: Gate current at 0.041000 mA, (heater 0.888000 A) ...
20:33:15: Gate current at 0.036000 mA, (heater 0.883000 A) ...
20:45:15: Gate current at 0.034000 mA, (heater 0.881000 A) ...
20:46:07: Activation SUCCESSFUL. Heater current is 0.881260 A.
20:46:08: End FT ECD cathode activation

ECD Calibration

After a bakeout and activation the ECD needs to be calibrated. Infuse Substance-P (2pmol/µl in 50:50 Methanol/Water with 0.1% formic acid) with ESI or NSI source and the following scan parameters:



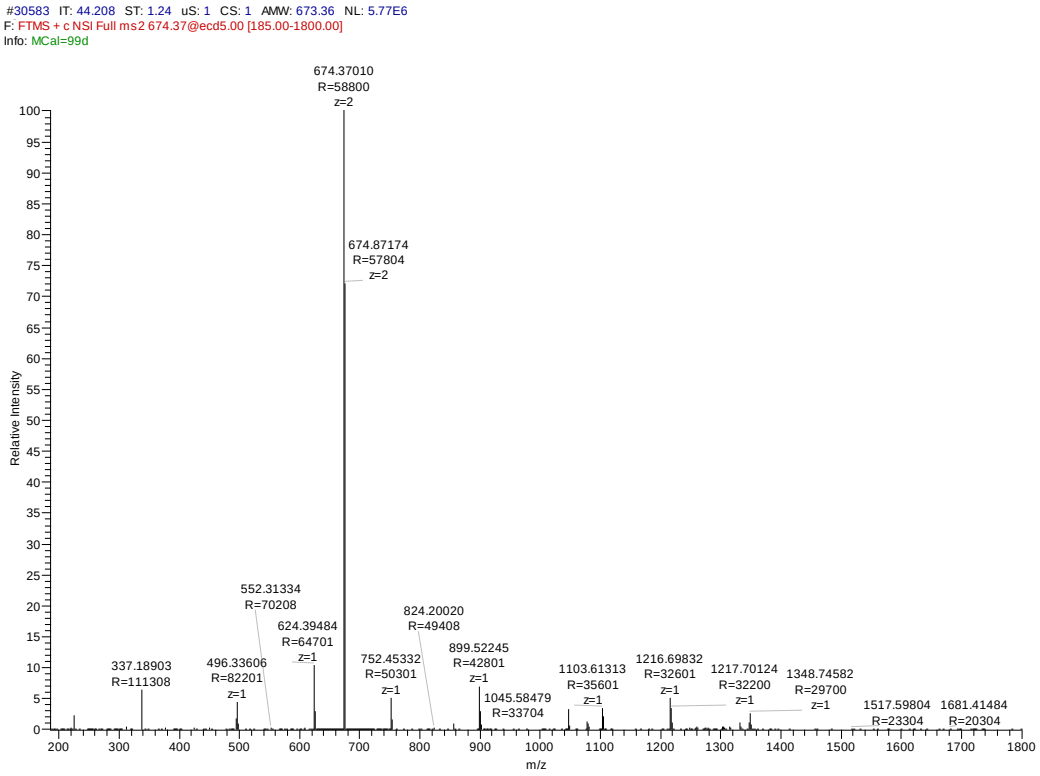
Next calibrate ECD Potentials and Timing (Calibrate/FT ECD/Calibrate):



15:05:09: Calibrating ECD potentials and timing.
15:05:09: Adjusting FT ECD delay
15:06:33: Previous Setting = 12.179775, New Setting = 12.179775
15:06:35: Adjusting FT ECD energy offset
15:07:31: Previous Setting = 3.687500, New Setting = 2.787500
15:07:32: Checking ECD efficiency...
15:07:52: Target 0.100000E6: ECD efficiency 33.300000 percent.
15:08:13: Target 0.200000E6: ECD efficiency 33.300000 percent.
15:08:35: Target 0.500000E6: ECD efficiency 16.600000 percent.
15:08:36: ECD efficiency at 33.300000 percent (target 0.100000E6).
15:08:36: ECD potentials and timing calibration SUCCESSFUL.

At any time you can also just run a check:

15:09:23: Checking ECD efficiency...
15:09:43: Target 0.100000E6: ECD efficiency 33.300000 percent.
15:10:04: Target 0.200000E6: ECD efficiency 29.000000 percent.
15:10:26: Target 0.500000E6: ECD efficiency 15.900000 percent.
15:10:27: ECD efficiency at 33.300000 percent (target 0.100000E6).
15:10:27: ECD potentials and timing calibration SUCCESSFUL.



Screen shot Substance P 2pmol/µl direct infusion, ECD on 674 ion

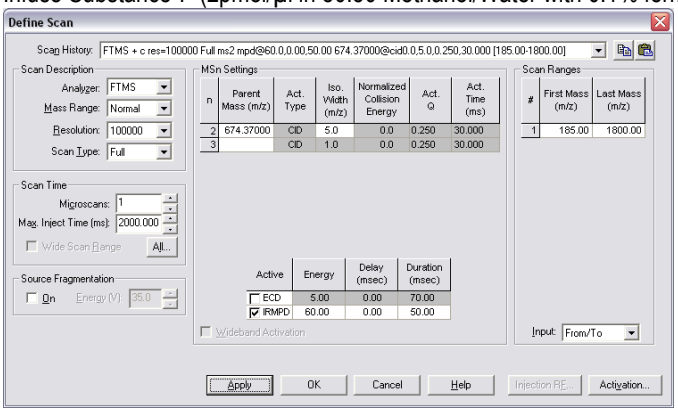
Note: Only calibrate Potentials and timing; don't calibrate Heater Current unless necessary

The ***Heater Current*** is calibrated as part of the ECD activation. But you can run a Heater Current Check (which is fast):

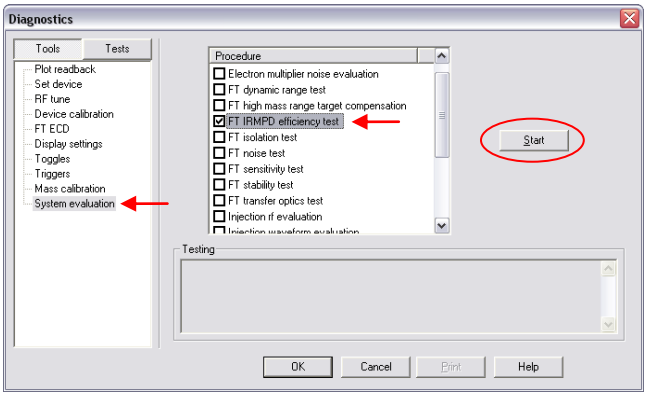
10:58:58: Checking calibration of ECD heater current.
10:58:58: Target gate current: 0.030000 mA
10:58:58: Actual gate current: 0.029000 mA
10:58:58: Calibrated heater current: 0.881260 A
10:58:58: Actual heater current: 0.958000 A
10:58:58: ECD heater current calibration OK.

IRMPD evaluation

Infuse Substance-P (2pmol/µl in 50:50 Methanol/Water with 0.1% formic acid) with ESI or NSI source and the following scan parameters:

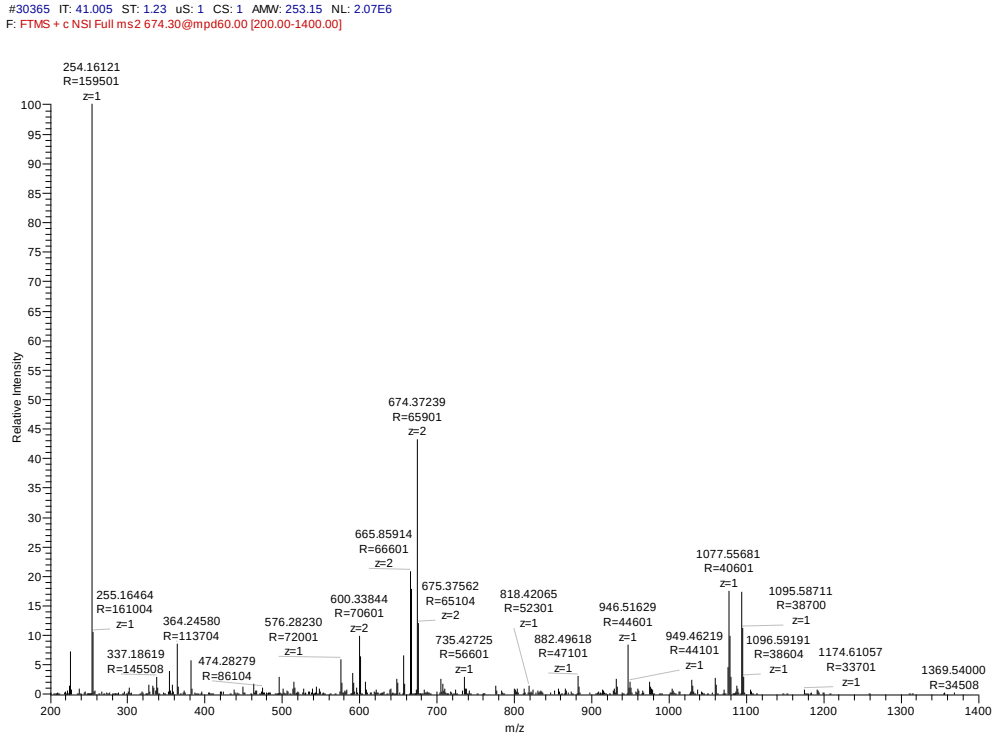


Then go to ***Diagnostics/System evaluation*** check ***FT IRMPD efficiency test*** and hit start:



11:41:18: Start FT IRMPD efficiency test
11:42:35: Target 0.100000E6: IRMPD efficiency 29.400000 percent.
11:43:52: Target 0.200000E6: IRMPD efficiency 38.500000 percent.
11:45:13: Target 0.500000E6: IRMPD efficiency 30.600000 percent.
11:46:19: Parent intensity below 50 % for IRMPD duration 54.493321 ms.
11:46:19: Result: IRMPD efficiency is at 38.5000 % for target 0.200000E6.
11:46:19: End FT IRMPD efficiency test

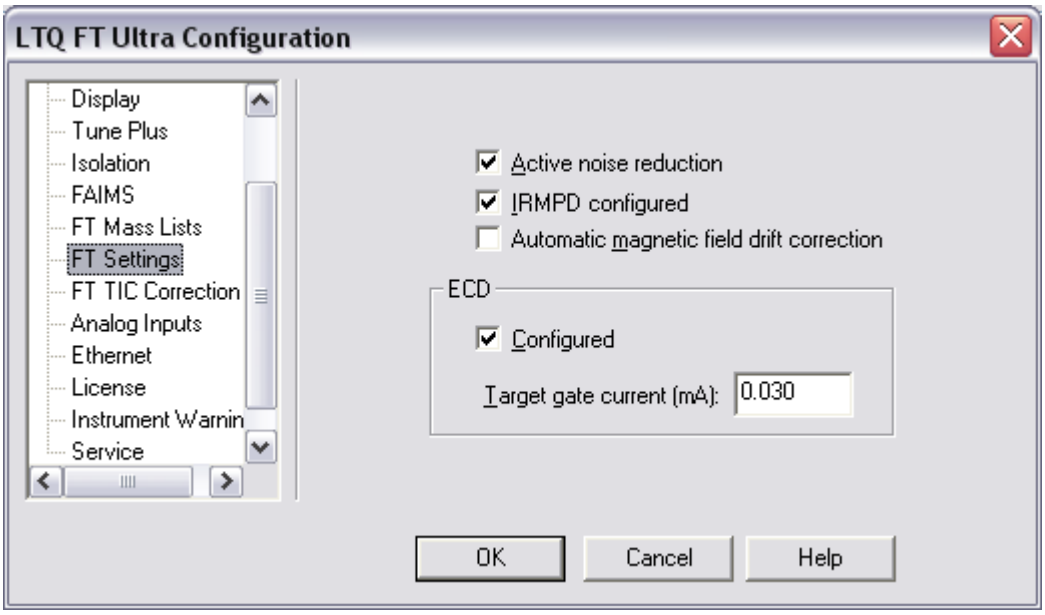
Specs:
Parent intensity below 50% in <100ms
IRMPD efficiency > 10% for target 0.5 E 6



Screenshot Substance P 2pmol/µl

ECD and IRMPD in "Instrument configuration"

E.g. after software upgrade make sure to configure the ECD and IRMPD, close Xcalibur and Tune window and open Instrument configuration window.



Troubleshooting:

ECD (and/or IRMPD) efficiency too low

NOTE: The ECD may require additional fine tuning once the system is set up for normal FT scanning and detecting. The IRMPD also may never show fragments if the FT cell is not properly optimized for ECD.

Once Offset, Trapping and Excite amplitudes are all calibrated and set to maximize the FT performance the ECD fragmentation may not be optimal.

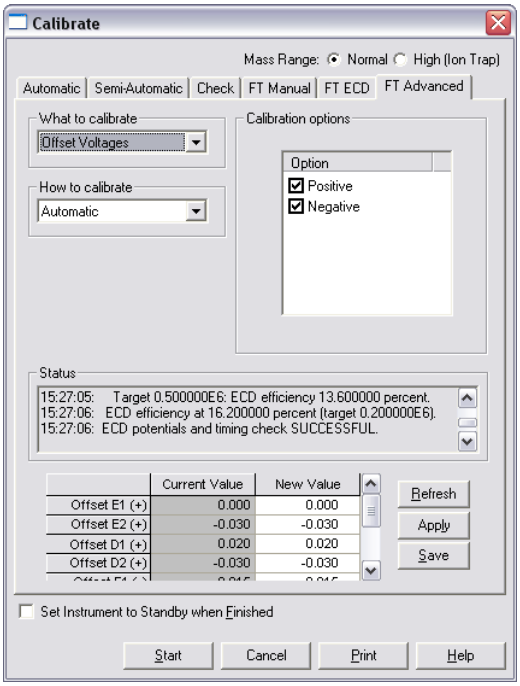
Check the ECD performance using Substance-P (alternatively it can be evaluated with MRFA 2+ ion at m/z 262). The evaluation should include all three FM scan ranges of the FT; FM>50, FM>98 & FM>190. If the ECD fragmentation is low (most likely in the FM>50 range) then it may be necessary to manually adjust the Offsets for the E1, E2, D1 & D2, even though the Offsets are not FM range dependent as per the settings they do affect the performance depending on the FM range selected. And even if the automatic Cali procedure may have been run for the offsets it may not be good for the ECD performance.

Note the current settings for Offsets, trapping and Excite amplitudes before starting the manual adjustment (I always take screen shots and copy/paste them to a word doc).

Look at the offset values and look for any values set with a negative value i.e. -0.05. If the D1 or D2 values are negative try these 1st one at a time. Simply use the same value but in a positive polarity. This should bring the ECD performance back in line (observe the fragment ion intensity and precursor intensity). Caution, if the value is a large # i.e. 0.04 or similar then by switching it to its opposite polarity may affect the normal full scan as it may now repel the ions away from the excite or detect plate. Try to use a value that is close to zero. Once adjusted you will have to go back and verify the FT full scan performance. You may have to see-saw back and for between the two to obtain optimal performance for both.

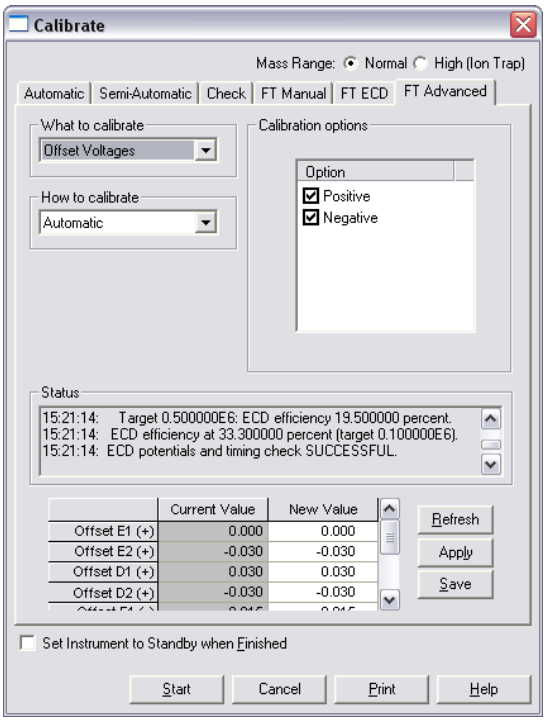
When you are done, save the new Offset values. And do a mass calibration.

Here is an example (NOTE the IRMPD performance improved as well):



15:26:02: Checking calibration of ECD potentials and timing.
15:26:23: Target 0.100000E6: ECD efficiency 13.100000 percent.
15:26:43: Target 0.200000E6: ECD efficiency 16.200000 percent.
15:27:05: Target 0.500000E6: ECD efficiency 13.600000 percent.
15:27:06: ECD efficiency at 16.200000 percent (target 0.200000E6).
15:27:06: ECD potentials and timing check SUCCESSFUL.

11:12:16: Start FT IRMPD efficiency test
11:12:16:
11:13:33: Target 0.100000E6: IRMPD efficiency 2.000000 percent.
11:14:50: Target 0.200000E6: IRMPD efficiency 2.500000 percent.
11:16:10: Target 0.500000E6: IRMPD efficiency 3.100000 percent.
11:16:10:
11:16:50: Parent intensity below 50 percent for IRMPD duration 18.138222 ms.
11:16:50: Result: IRMPD efficiency is at 3.100000 percent for target 0.500000E6.
11:16:51: End FT IRMPD efficiency test



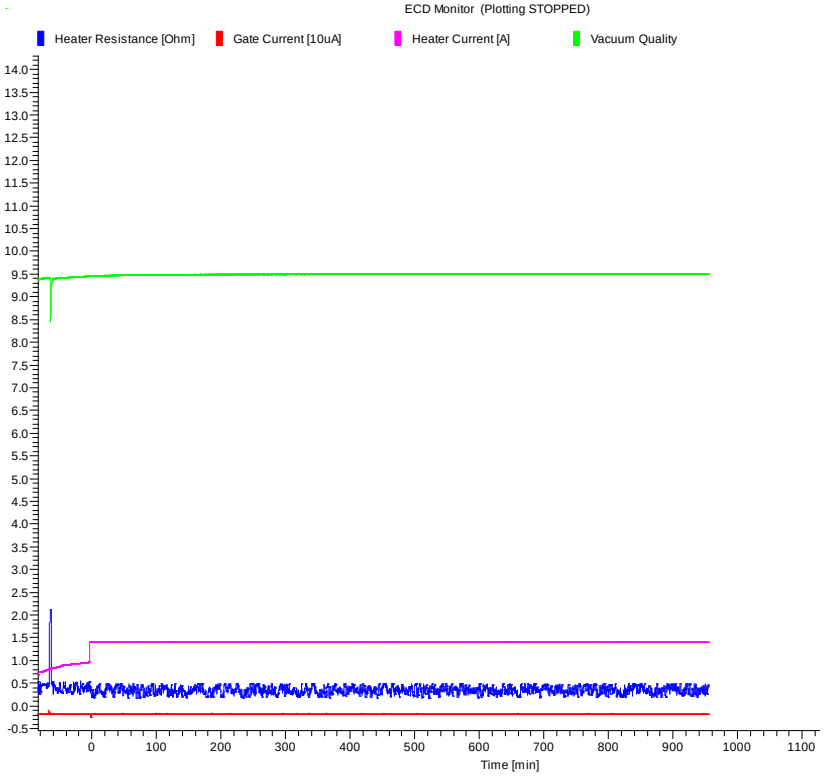
15:20:11: Checking calibration of ECD potentials and timing.
15:20:32: Target 0.100000E6: ECD efficiency 33.300000 percent.
15:20:52: Target 0.200000E6: ECD efficiency 29.600000 percent.
15:21:14: Target 0.500000E6: ECD efficiency 19.500000 percent.
15:21:14: ECD efficiency at 33.300000 percent (target 0.100000E6).
15:21:14: ECD potentials and timing check SUCCESSFUL.

15:50:02: Start FT IRMPD efficiency test
15:50:02:
15:51:21: Target 0.100000E6: IRMPD efficiency 54.100000 percent.
15:52:39: Target 0.200000E6: IRMPD efficiency 53.300000 percent.
15:54:02: Target 0.500000E6: IRMPD efficiency 27.500000 percent.
15:54:02:
15:54:44: Parent intensity below 50 percent for IRMPD duration 19.545133 ms.
15:54:44: Result: IRMPD efficiency is at 54.100000 percent for target 0.100000E6.
15:54:44: End FT IRMPD efficiency test

ECD won't stay in "on" position

Often when the ECD was in standby or off, it won't stay on when switching it on.
When switching the ECD on it will try to warm up but after a few minutes will return to standby. Typically running the ECD activation will solve this problem. After the activation is complete the ECD should remain in the on position.

During the activation if the (gate current) red line drops off eary or doesn't raise up at all you likely have a problem with the ECD. I don't know the official lifetime of the ECD cathode. But repeated venting of the instrument when the cathode is warm (ON) will ultimately cause it to burn out. Note we had a couple of unplanned power outages and finally our cathode burnt out when the instrument vented again with the ECD turned on. After pumping down and bakeout we ran an activation. This is what the activation plot looked like:



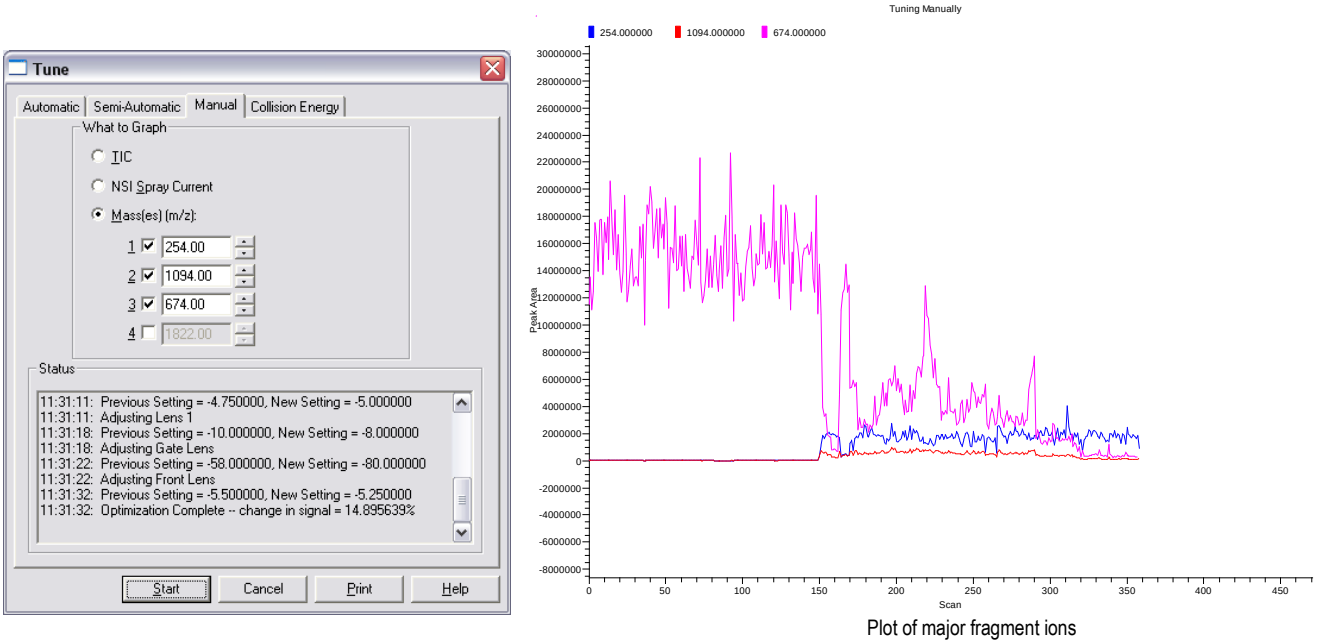
We decided to have a Thermo engineer replace the ECD cathode. You may be able to replace it yourself; however the price for replacement part is high enough to warrant the cost for labor… Just as a side note the first ECD the engineer put in didn't work, he then replaced it with another new one and that one worked, so if you do it yourself you'll have to fight with Thermo explaining that you didn't break it during install.…

IRMPD not ready

There are safety interlocks that prevent the IRMPD laser to turn on when the LTQ table is moved out of the magnet. Occasionally when we move the LTQ table back inside the magnet those interlocks won't close preventing the laser to turn on. We found a couple of ground wires (green/yellow cables attached to the chassis) that would get pinched between the rail and the cassis, creating a distance just long enough to keep the interlocks open. After moving those cables, the LTQ table now slides in all the way and the interlocks close properly and the laser comes on every time.

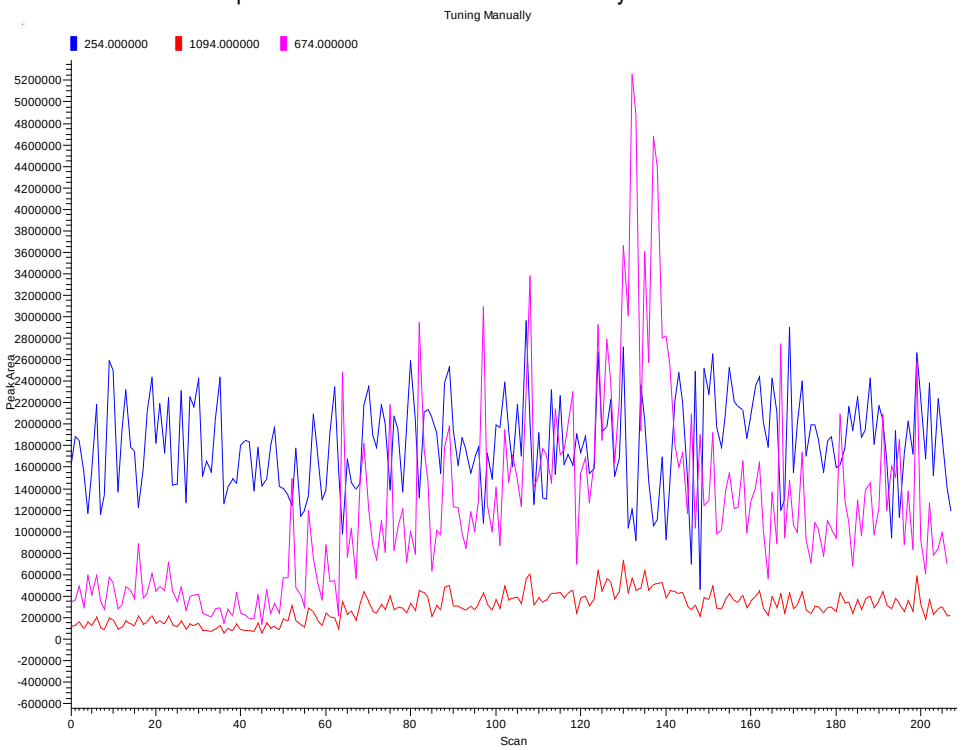
IRMPD is misaligned

If the IRMPD efficiency is way too low and adjusting the offsets as described above didn't improve the IRMPD performance, you can try to manually adjusting the mirrors for the laser:
If the laser ever gets misaligned, readjust it with screws hidden under black rubber covers on the back side of the instrument. Watch the 254, 1094 and 674 peaks (plot masses in graph view) and try to maximize both (the 674 may decrease as the 254 and 1094 increase).
If the laser is way off you may have to use the target for a coarse realignment: pull the LTQ table out of the magnet and take off the black tube that is attached to the ECD. Attach the target in front of the whole of the magnet with the 4 thumbscrews. Plug in the power adapter for the laser in the back of the instrument and use the guide light (note there is an on/off switch) to adjust the laser. Put everything back together and fine adjust as described above. And run efficiency test again. We typically get >20%.

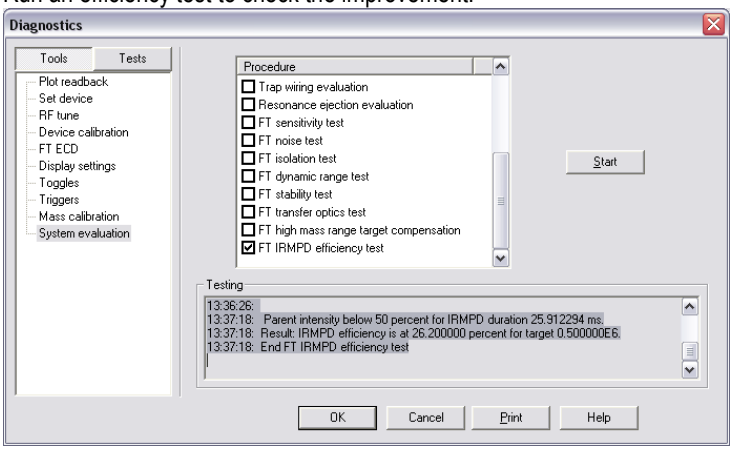


Plot of major fragment ions

Restart the Manual Tune plot to better see the details if necessary:



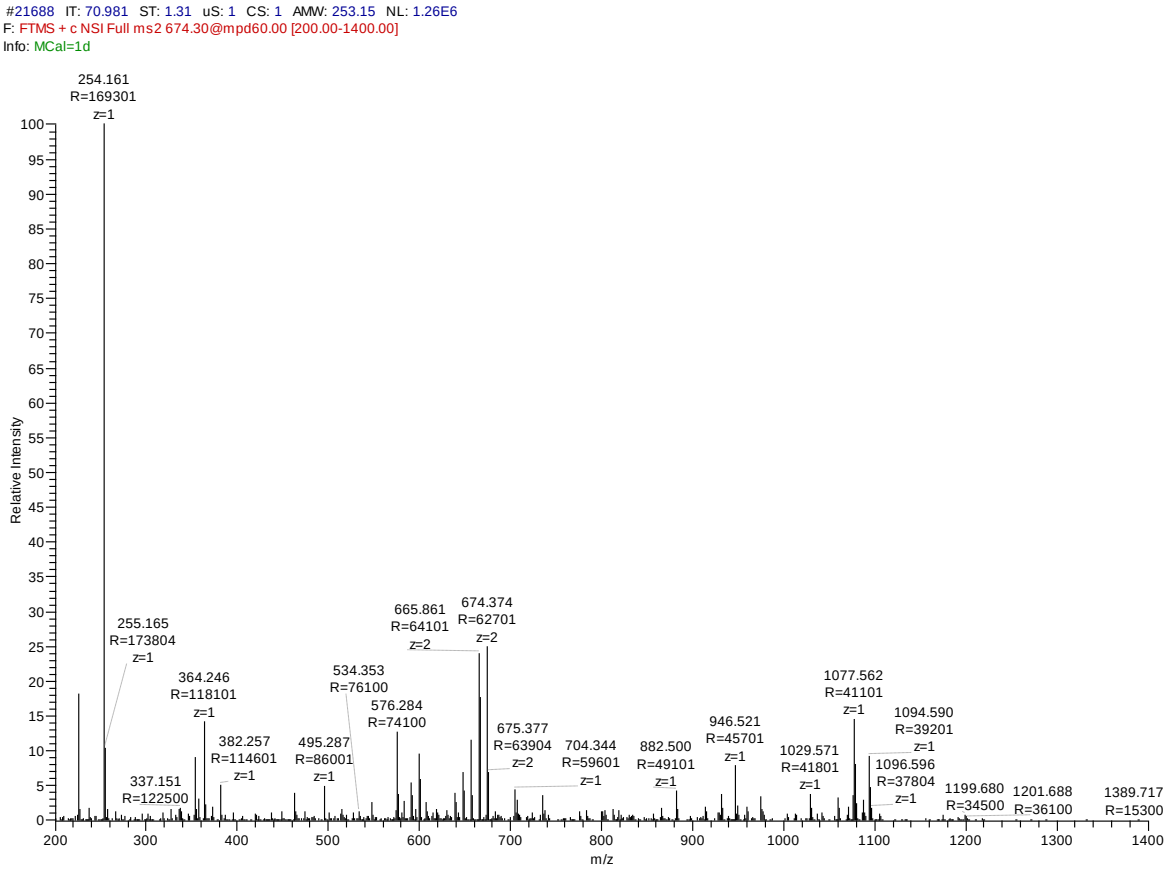
Run an efficiency test to check the improvement:



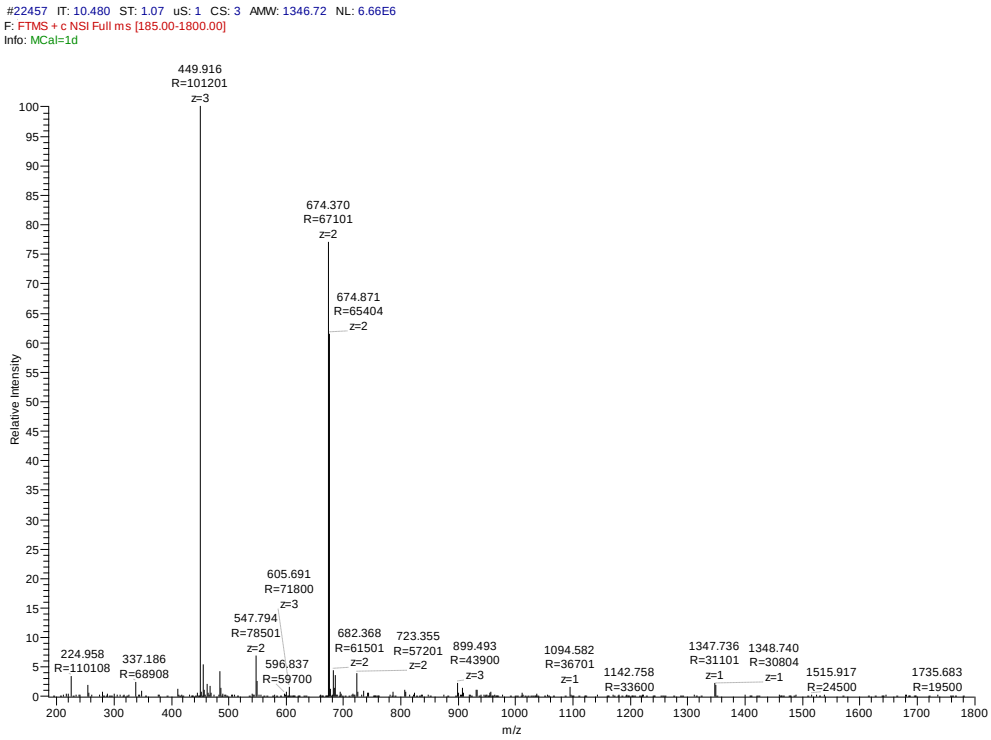
13:31:58: Start FT IRMPD efficiency test
13:31:58:
13:33:26: Target 0.100000E6: IRMPD efficiency 23.200000 percent.
13:33:26:
13:34:55: Target 0.200000E6: IRMPD efficiency 22.900000 percent.
13:34:55:
13:36:26: Target 0.500000E6: IRMPD efficiency 26.200000 percent.
13:36:26:
13:37:18: Parent intensity below 50 percent for IRMPD duration 25.912294 ms.
13:37:18: Result: IRMPD efficiency is at 26.200000 percent for target 0.500000E6.
13:37:18: End FT IRMPD efficiency test

Specs are:
Parent intensity below 50% in <100ms
IRMPD efficiency > 10% for target 0.500000E6

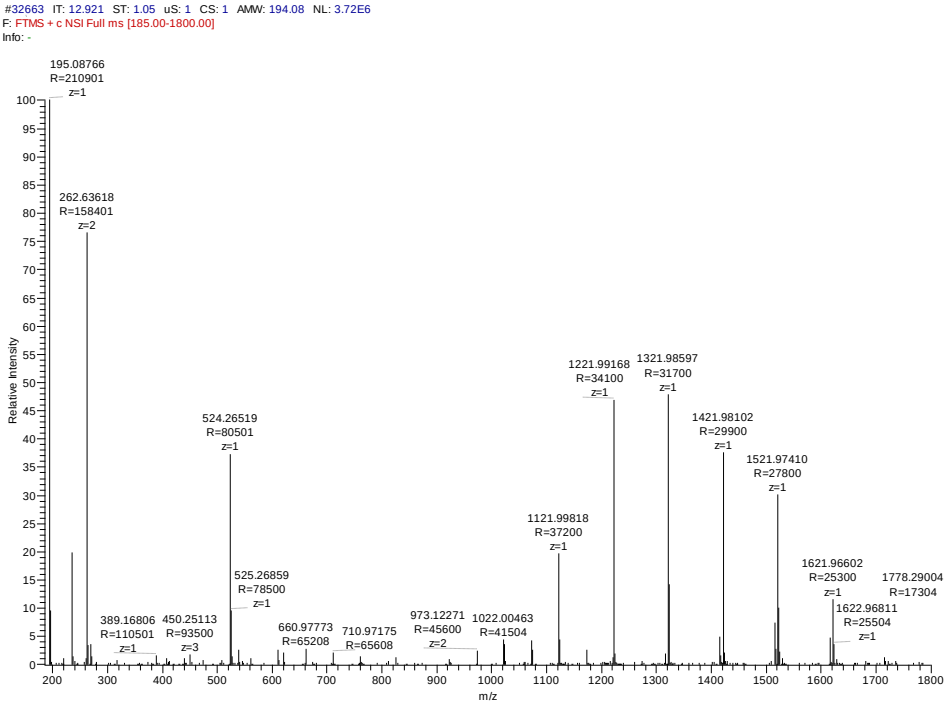
I'm happy with that! But adjusting the offsets may improve this more.



IRMPD screenshot of Substance P (2pmol/µl) infused with NSI source



Screenshot of 2pmol/µl substance P direct infusion with NSI source
Full scan 180-1800m/z



Screen shot of standard calmix, NSI source, FT full scan