

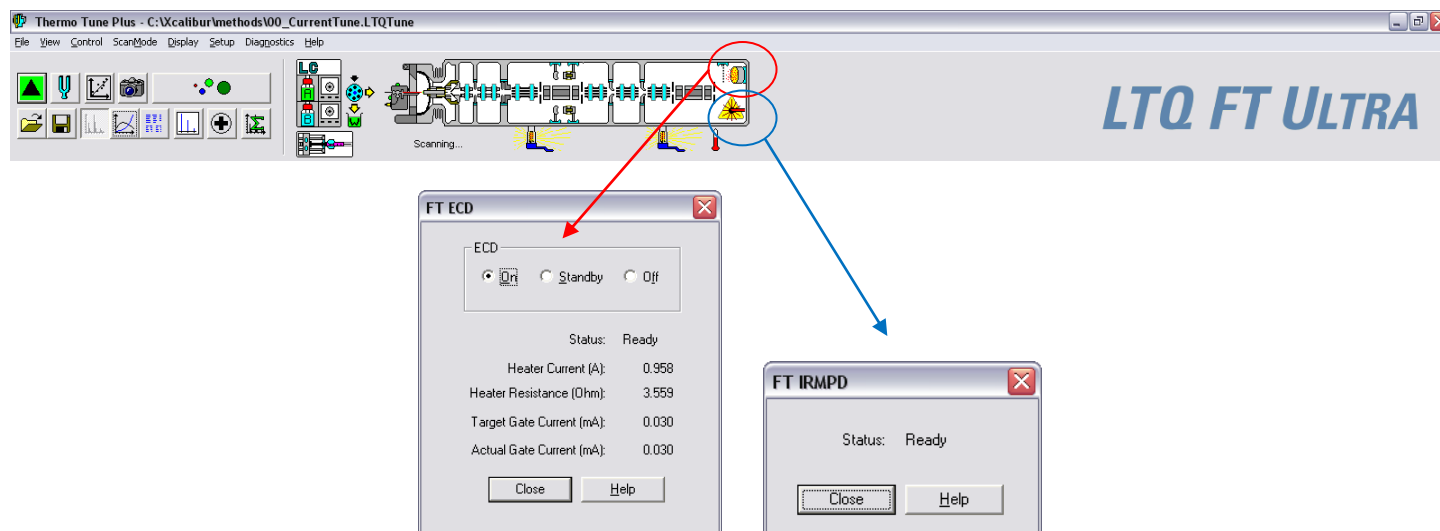
## **ECD and IRMPD optimization**

Note this document is intended to help guide you through some basic manual ECD and IRMPD optimization.

For a comprehensive 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.

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## **ECD/IRMPD windows**



Make sure the ECD is in "On" and the IRMPD is ready. While 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. If the ECD is in "Standby" or "Off", turn it on. It will show heating and eventually switch to "On", this may take ~1hr. If it goes back to "Standby" try ECD activation (see LTQ\_FTUltra\_ECD\_IRMPD\_maintenance).

If you want to check the instruments performance infuse Substance-P and check the ECD calibration and IRMPD efficiency as described in LTQ\_FTUltra\_ECD\_IRMPD\_maintenance. Both checks will give you the efficiency at three different target values. Use the target value with the highest efficiency to start the optimization.

**Optimization from Tune window**

Infuse Substance-P (2pmol/μl in 50:50 Methanol/Water with 0.1% formic acid) or your sample of interest with following scan params:  
 We use the direct infusion line with our NSI source, using the syringe pump in the LTQ at 3-5μl/min. Note the Isolation Width should be ~3-5 m/z

**Define Scan**

Scan History: FTMS + c res=100000 Full ms 2 674.37000@cid0.0,3.0,0.250,30.000 [185.00-1800.00]

Scan Description: Analyzer: FTMS, Mass Range: Normal, Resolution: 100000, Scan Type: Full

Scan Time: Microscans: 1, Max. Inject Time (ms): 2000.000, Wide Scan Range: [ ]

Source Fragmentation: On, Energy (V): 35.0

MSn Settings:

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2	674.37000	CID	3.0	0.0	0.250	30.000
3		CID	1.0	0.0	0.250	30.000

Scan Ranges:

#	First Mass (m/z)	Last Mass (m/z)
1	185.00	1800.00

Active Fragmentation:

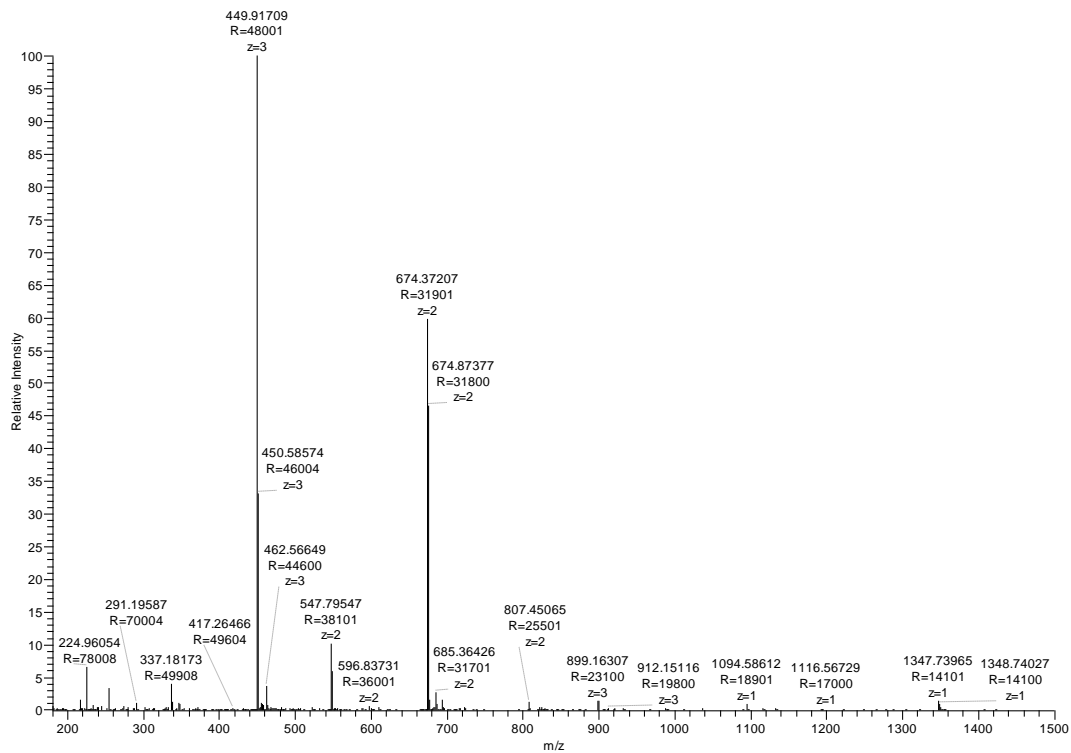
Active	Energy	Delay (msec)	Duration (msec)
<input checked="" type="checkbox"/> ECD	5.00	0.00	70.00
<input type="checkbox"/> IRMPD	60.00	0.00	50.00

Wideband Activation: [ ]

Input: From/To

Buttons: Apply, OK, Cancel, Help, Injection PE..., Activation...

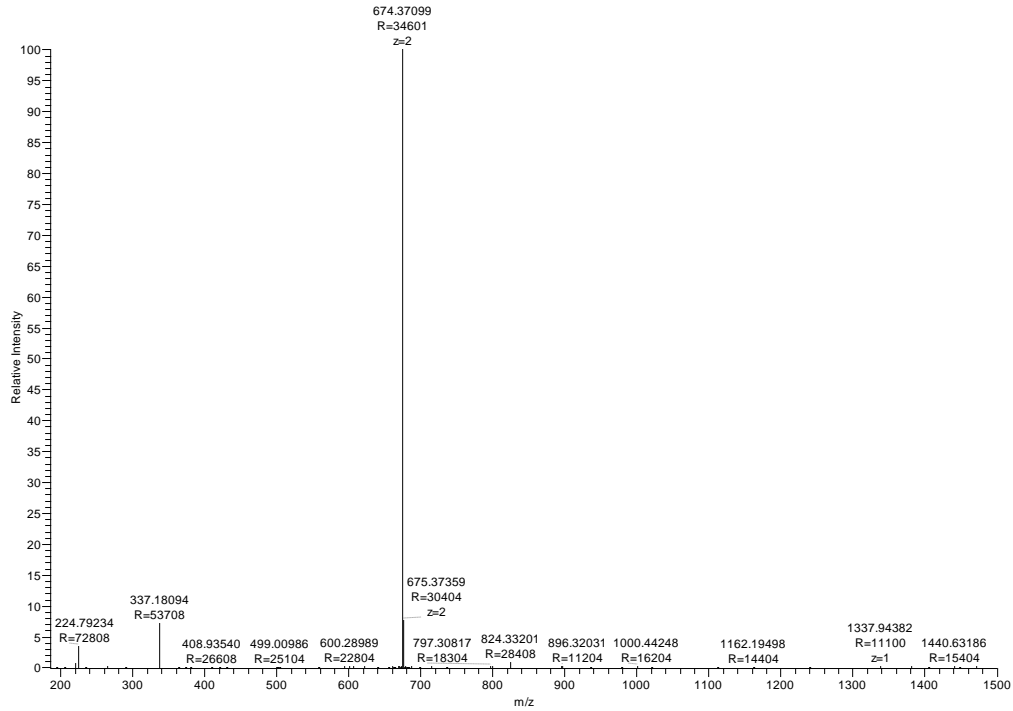
#39144 IT: 31.040 ST: 0.72 uS: 1 CS: 3 AMW: 1346.73 NL: 6.05E6  
 F: FTMS + c NSI Full ms [180.00-1500.00]



Substance P (2pmol/μl in 50:50 Methanol/Water with 0.1% formic acid), Full scan m/z 180-1500

To isolate your parent enter the parent mass m/z, and isolation width of 3-5 m/z and a collision energy of 0 and don't check ECD or IRMPD. This way you should see the isolated parent without any fragmentation. (the two ions at m/z 224 and 337 are harmonics of the parent)

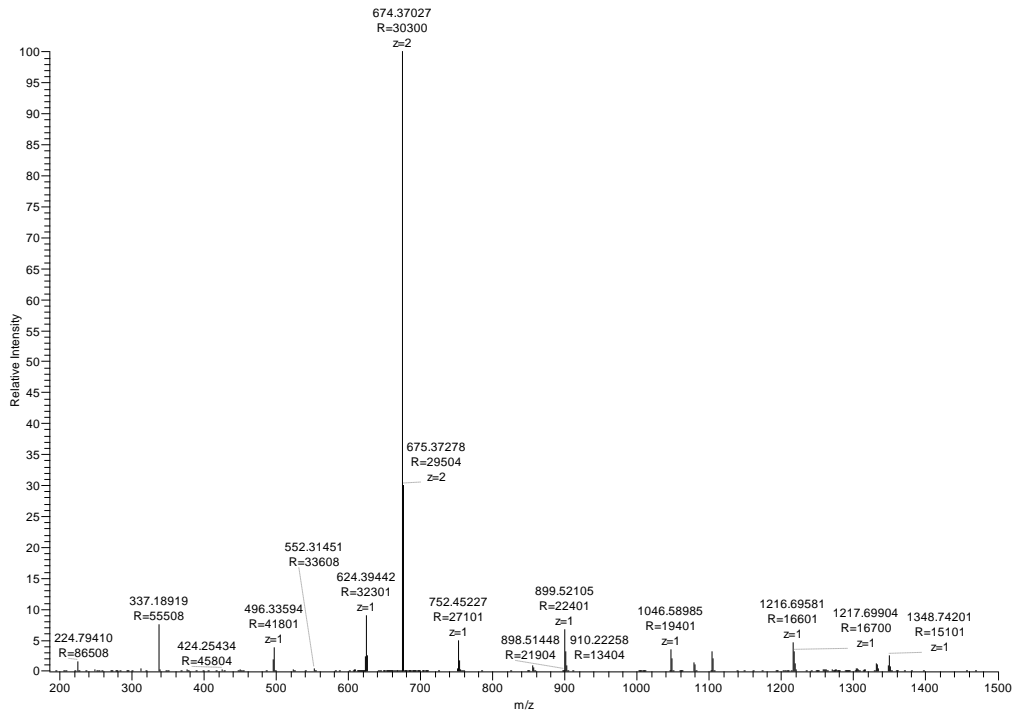
#40763 IT: 32.023 ST: 0.83 uS: 1 CS: 2 AMW: 1346.73 NL: 1.82E6  
 F: FTMS + c NSI Full ms2 674.37@cid0.00 [185.00-1500.00]



Isolating m/z 674.37 with isolation width 3 m/z

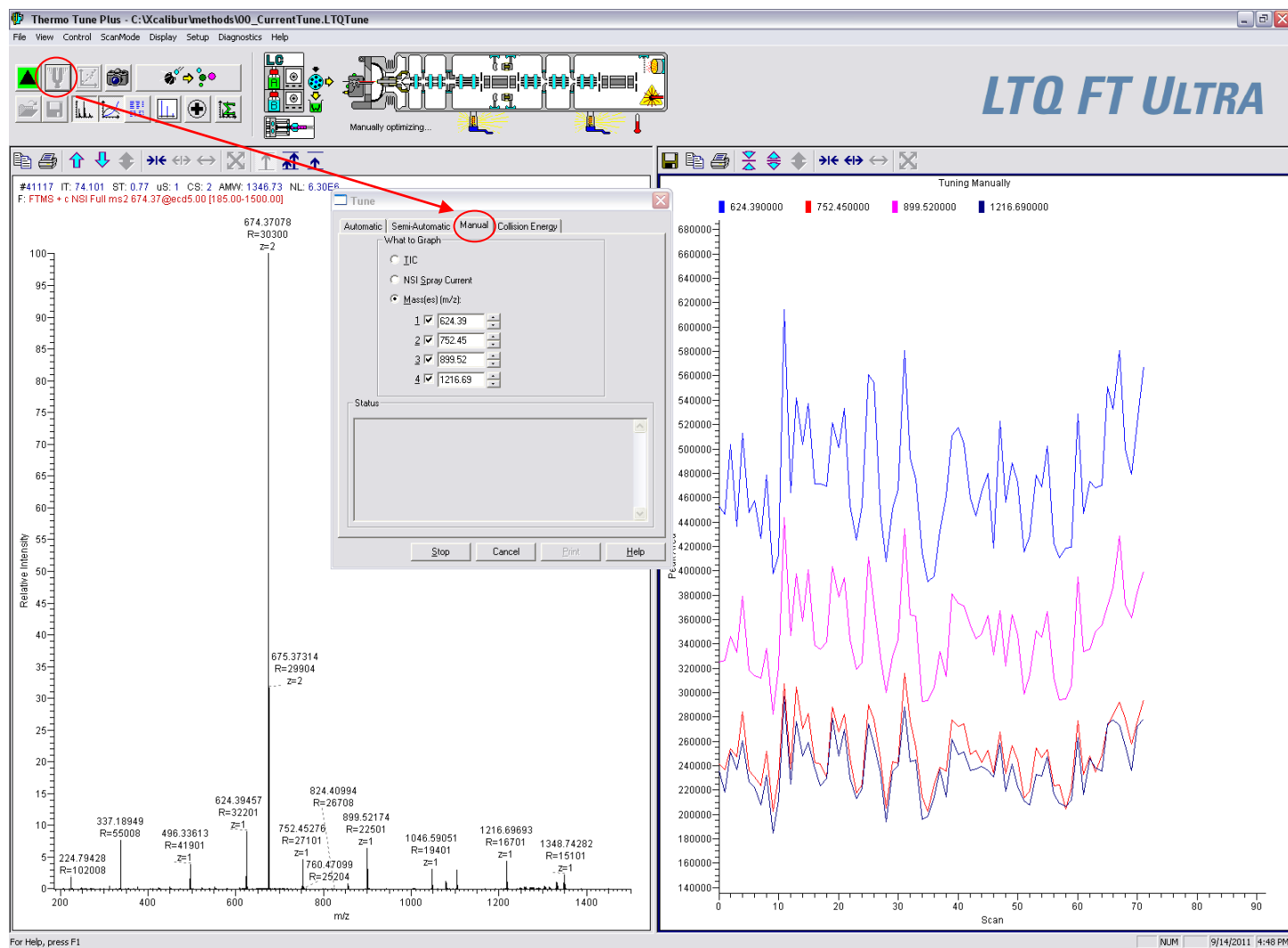
Now check the ECD checkbox and you should see fragmentation of your parent:

#40847 IT: 80.223 ST: 0.91 uS: 1 CS: 2 AMW: 1346.73 NL: 5.06E6  
 F: FTMS + c NSI Full ms2 674.37@ecd5.00 [185.00-1500.00]

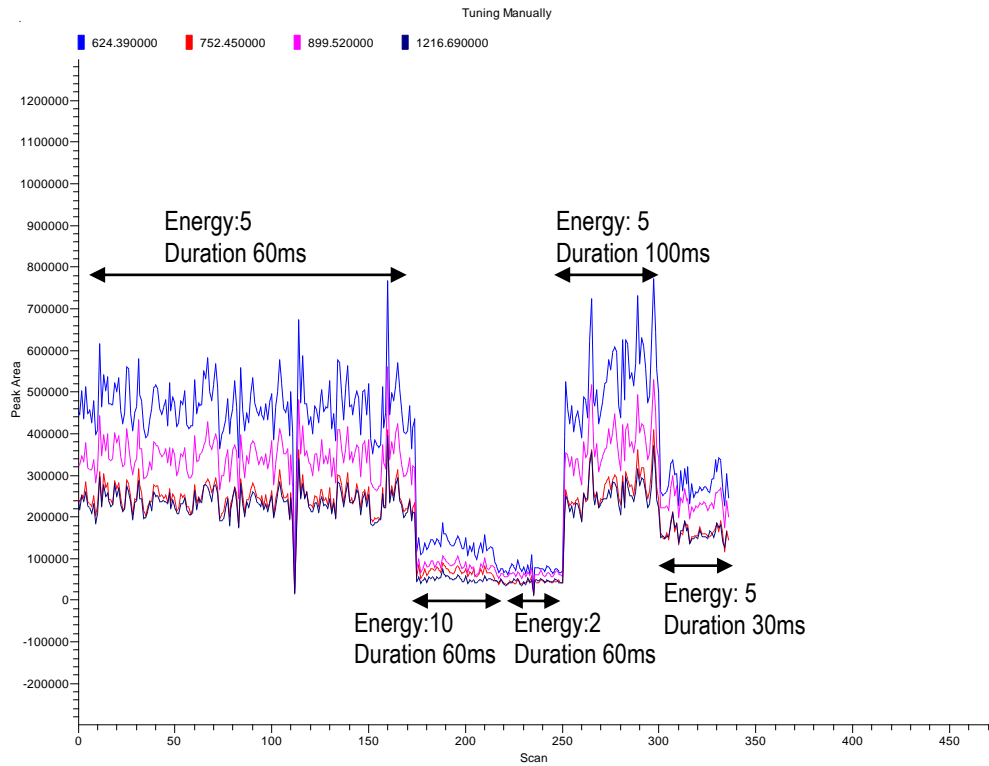


ECD on 674.37, energy 5, duration 60ms

Now plot TIC or some fragment ions or the parent plus 3 fragment ions etc (you can plot up to 4 m/z values), click on the Tune fork in the Tune window, go to the Manual tab and enter the masses you want to plot, then hit start:



Now start playing with the ECD energy, duration and target values until you maximize the fragmentation for your compound. I always start with the default values for the energy and duration. For the target I start with the target that resulted in the best efficiency during the calibration. Below is a screen shot for various energy and duration values while plotting four Substance-P fragments.



Substance-P ECD fragments at different energy and duration, fixed target



Substance-P ECD fragments at different target values, with fixed energy and duration

**IRMPD optimization**

Repeat the same thing for the IRMPD, infuse sample of interest and start with the default params.

**Define Scan**

Scan History: FTMS + c res=100000 Full ms2 mpd@60.0.0.0.50.0.0.674.37000@csd0.0.5.0.0.250.30.000 [185.00-1800.00]

Scan Description: Analyzer: FTMS, Mass Range: Normal, Resolution: 100000, Scan Type: Full

Scan Time: Microscans: 1, Mag. Inject Time (ms): 2000.000

Source Fragmentation: Energy (V): 35.0

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2	674.37000	CD	5.0	0.0	0.250	30.000
3		CD	1.0	0.0	0.250	30.000

#	First Mass (m/z)	Last Mass (m/z)
1	185.00	1800.00

Active	Energy	Delay (msec)	Duration (msec)
<input type="checkbox"/>	ECD	5.00	70.00
<input checked="" type="checkbox"/>	IRMPD	60.00	50.00

Input: From/To

**Thermo Tune Plus - C:\Xcalibur\Methods\00\_CurrentTune.LTQTune**

File View Control ScanMode Display Setup Diagnostics Help

Scanning...

**LTQ FT ULTRA**

#42551 IT: 18.458 ST: 0.85 uS: 1 CS: 1 AMV: 253.15 NL: 2.0956  
F: FTMS + c NSI Full ms2 674.37@mpd60.00 [185.00-1500.00]

254.16114  
R=90501  
z=1

364.24566 R=61901 z=1  
463.19780 R=48601 z=1  
576.28199 R=38601 z=1  
665.85847 R=33701 z=2  
674.37209 R=34101 z=1  
824.40155 R=27308 z=1  
946.51594 R=23601 z=1  
1077.55549 R=21001 z=1  
1094.58314 R=21401 z=1  
1105.55461 R=24904 z=1  
1369.97923 R=11604 z=1

**Tune**

Automatic | Semi-Automatic | **Manual** | Collision Energy

What to Graph:

- TIC
- NSI Spray Current
- Mass(es) (m/z):
  - 1  495.28
  - 2  576.28
  - 3  946.51
  - 4  1077.55

Status:

Start Cancel Print Help

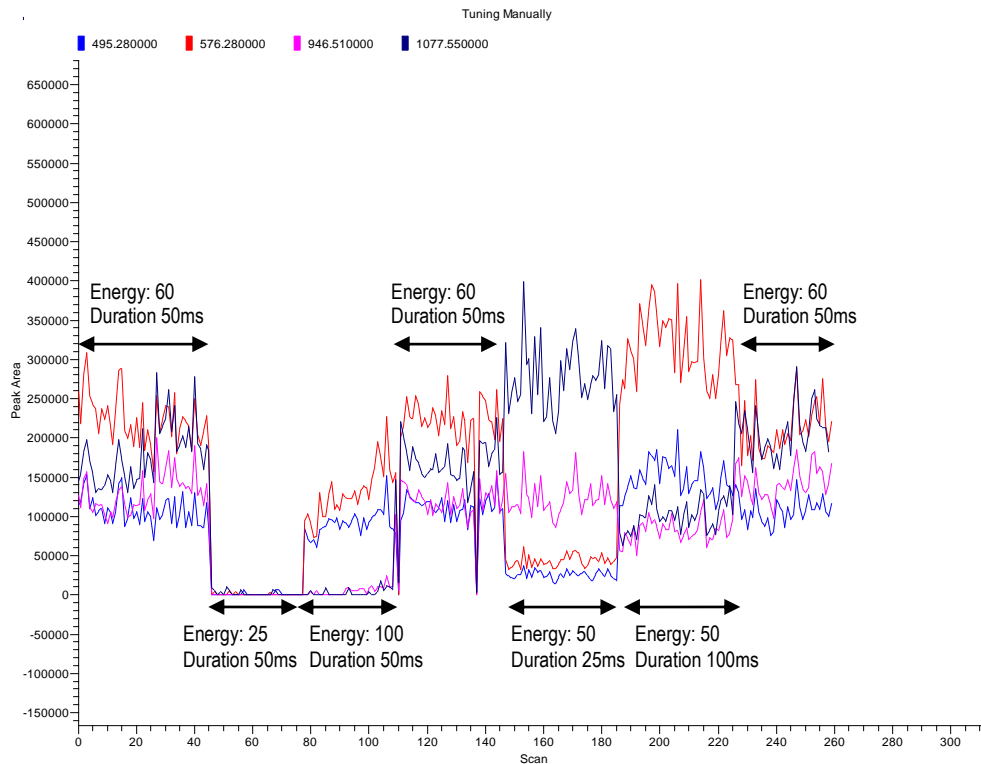
Tuning Manually

495.280000 576.280000 946.510000 1077.550000

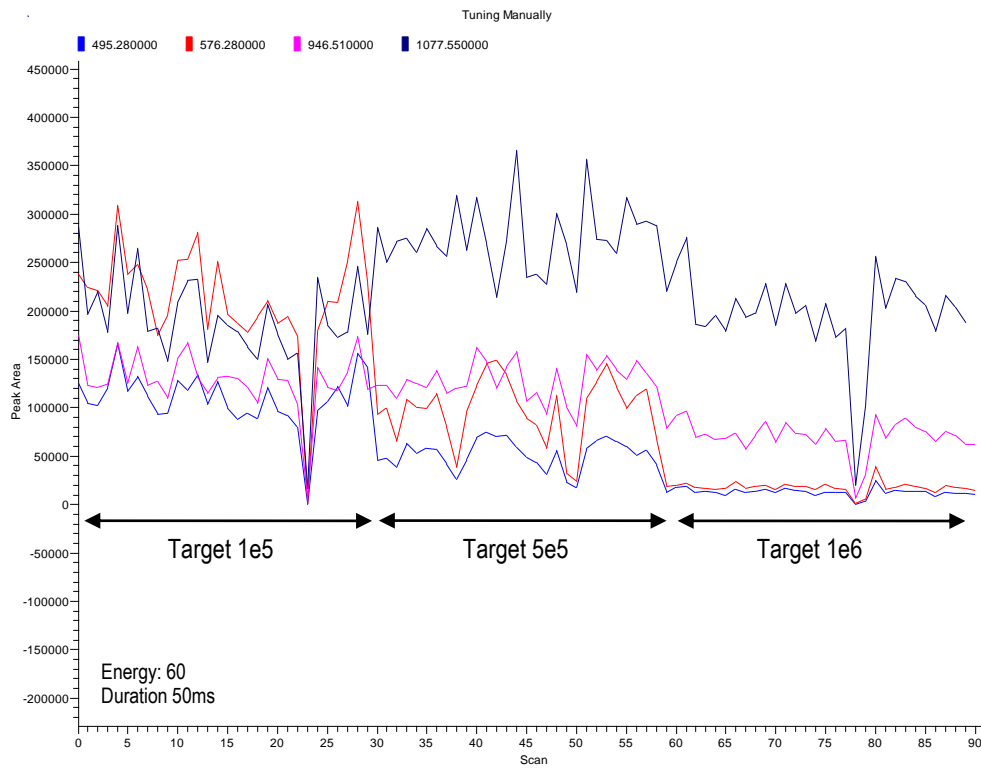
Peak Area

Scan

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Substance-P IRMPD fragments at different energy and duration, fixed target



Substance-P IRMPD fragments at different target values, with fixed energy and duration