

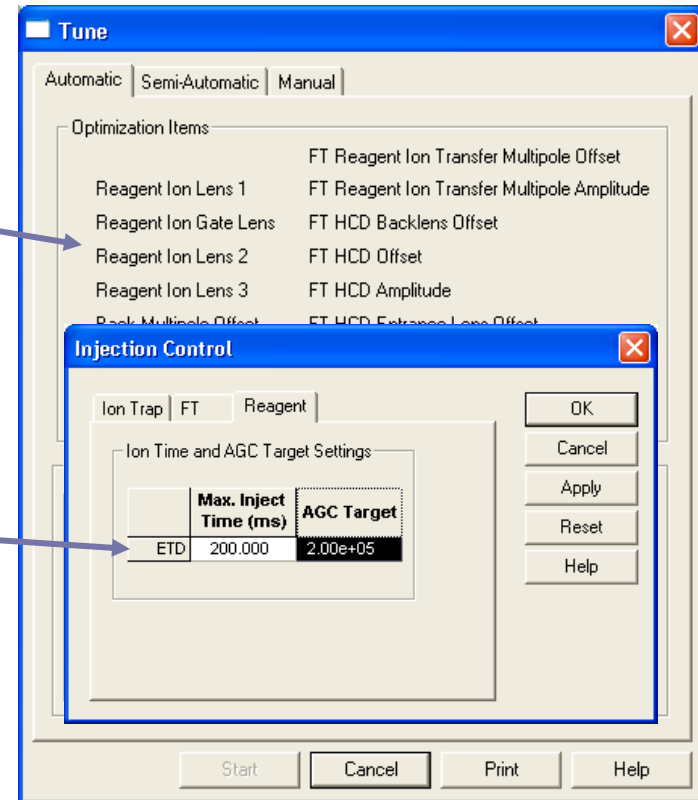
Setting up Tune files

Important Note: When you tune ETD, you must save your tune file.

- Tune settings are only saved to the individual tune file.
- If you change your source (HESI, ESI, nanospray), the tune file changes to the last opened tune file with that source. Because of this, you need to tune, ETD or otherwise, with the source you plan to use for running experiments or methods, and be sure to save the Tune file once tuning is complete.

Tune file parameters of consequence for ETD

- Tuning for 202 (ion optics)
 - The ion optics tune values are saved in your tune file. You need to tune 202 for the tune file you wish to use for your ETD experiment
 - NOTE: You need to retune if HCD gas was turned off to run experiments (for intact proteins on OT platforms)
- Reagent AGC target 1-2 e5
 - Max. injection time could set to 200 (instead of 50) so that 202 signal drop won't stop LC run



Optimizing the ETD Reaction Time if Necessary.

- The default ETD reaction time is 50 -100 ms. Using charge state dependent ETD methods, it works well for most of peptides. Therefore, it is a good choice for a complex, unknown sample.
- If necessary, the ETD reaction time can be optimized for a particular precursor ion. To do so, 1st setup the scan. It is necessary to infuse the analyte of interest for this experiment. Shown are the parameters for Angiotensin I.

1. Enter mass of ion of interest

Scan History: ITMS + c Full ms [150.00-2000.00]

Scan Description

Mass Range: Normal

Scan Rate: Normal

Scan Type: Full

Scan Time

Microscans: 1

Max. Inject Time (ms): 100.000

Source Fragmentation

Qn 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	433.00	ETD	2.0	35.0	0.250	100.00
3		CID	1.0	0.0	0.250	30.000

Scan Ranges

#	First Mass (m/z)	Last Mass (m/z)
1	115.00	2000.00

Input: From/To

Apply OK Cancel Help Injection RE... Activation...

4. Click to apply

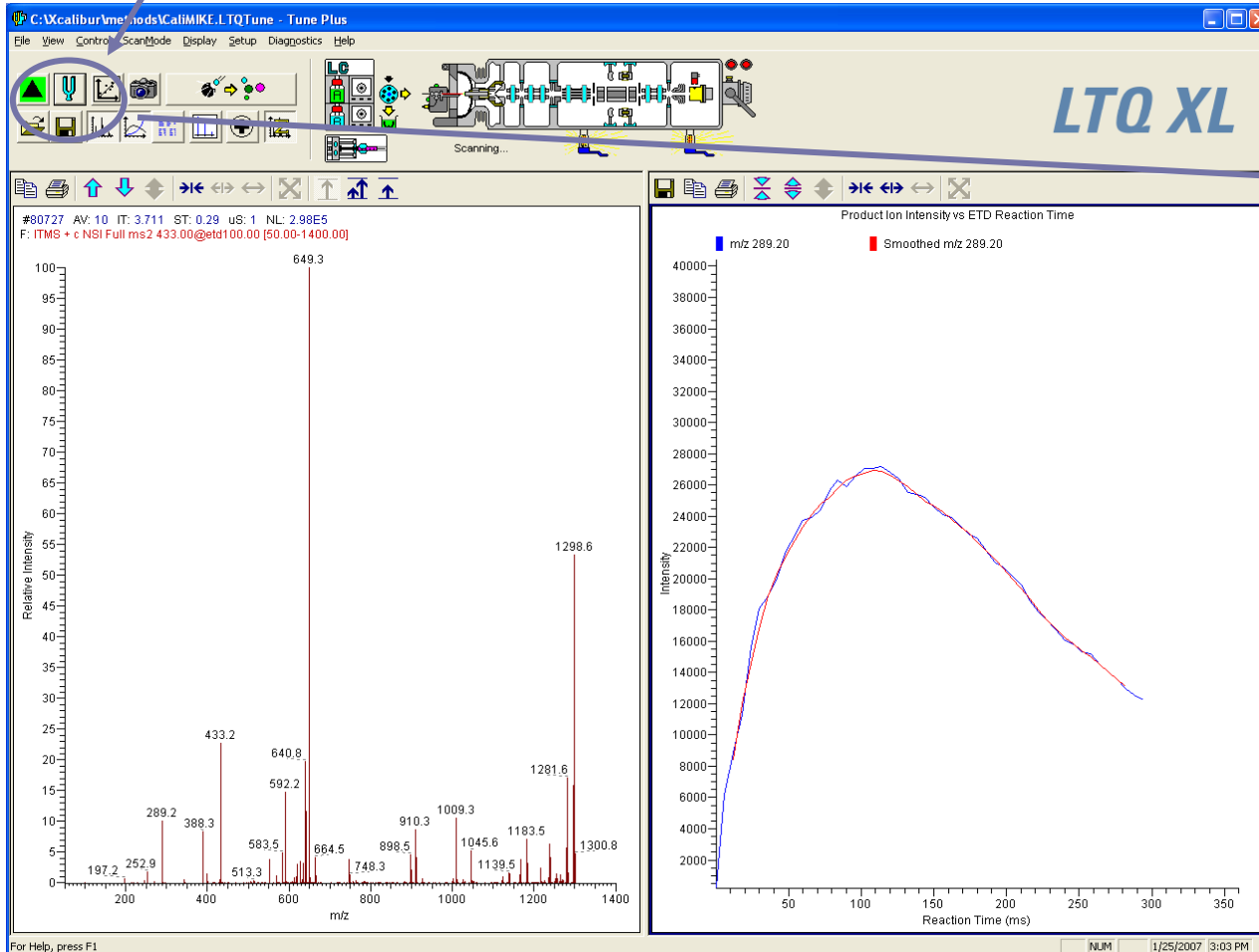
2. Select ETD fragmentation

3. Select ETD Reaction Time

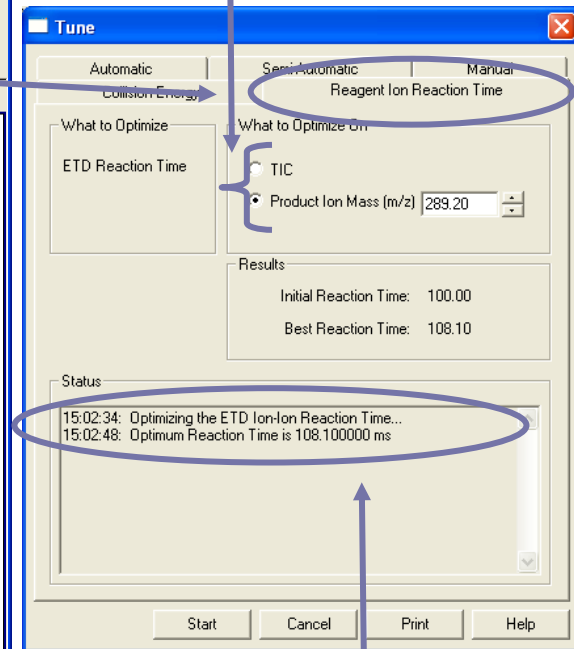
Tuning the ETD Reaction Time.

Note: the default value (50 -100 ms) works well for most peptides

1. Click the Tune icon



2. Select TIC or type mass of product ion



3. Click start

4. Result is displayed here