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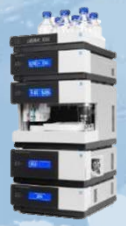
2016 iQuan Series: Pesticide Quantitation on the Thermo Scientific™ TSQ Quantiva™

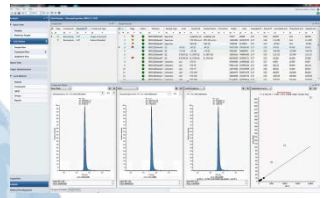
Craig Dufresne and Kevin McHale, Content Creators

The Whole Workflow …

Efficiency in sample preparation



Outstanding resolution from UHPLC



One software for both HRAM and QqQ

Service support to ensure increased uptime

Sample Prep & consumables → **LC** → **MS** → **SW** → **Apps Support** → **Service Support**

Robust, sensitive, Triple Quadrupole MS

From method development to training

- Routine, affordable quantitation
- Either part of an existing workflow, or used as a comprehensive workflow

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Expectation from LC-MS Technology

Hundreds of pesticides identified and quantified in one run

A comprehensive platform solution with hardware and software

Demand for lower LLOQ with higher selectivity, specificity, and confidence

Easier, faster option to final results while reducing cost/sample

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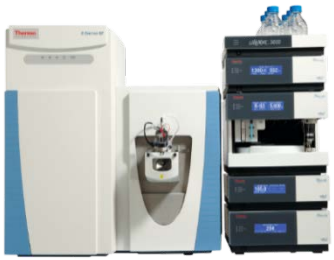

Demand for lower LLOQ with higher selectivity, specificity, and confidence

Easier, faster option to final results while reducing cost/sample

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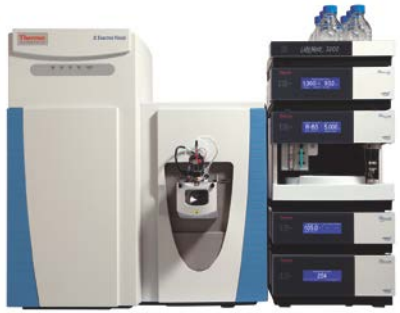
Pesticide Explorer – One Problem…Multiple Solutions!

Thermo Scientific™ TSQ Endura™ Triple Quadrupole MS	Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole MS	Thermo Scientific™ Q Exactive™ Focus MS	Thermo Scientific™ Q Exactive ™ Focus MS
Standard QUAN	Premium QUAN	HRAM QUAN	HRAM SCREEN QUAN



OPTON-30425	OPTON-30427

- Includes Software, Libraries, Methods, and Documentation, and Basic Pesticide Standards
- Optional Extended and Recommended Pesticides can be purchased
- All the sample prep materials can be purchased too

Analytical Challenges of Residue Analysis

- Sample variability (matrix)
- Compound characteristics
- Number of samples
- Number of analytes monitored
- Low levels controlled (<10 ng/g)
- Fast response required

Goals of Today's Webinar

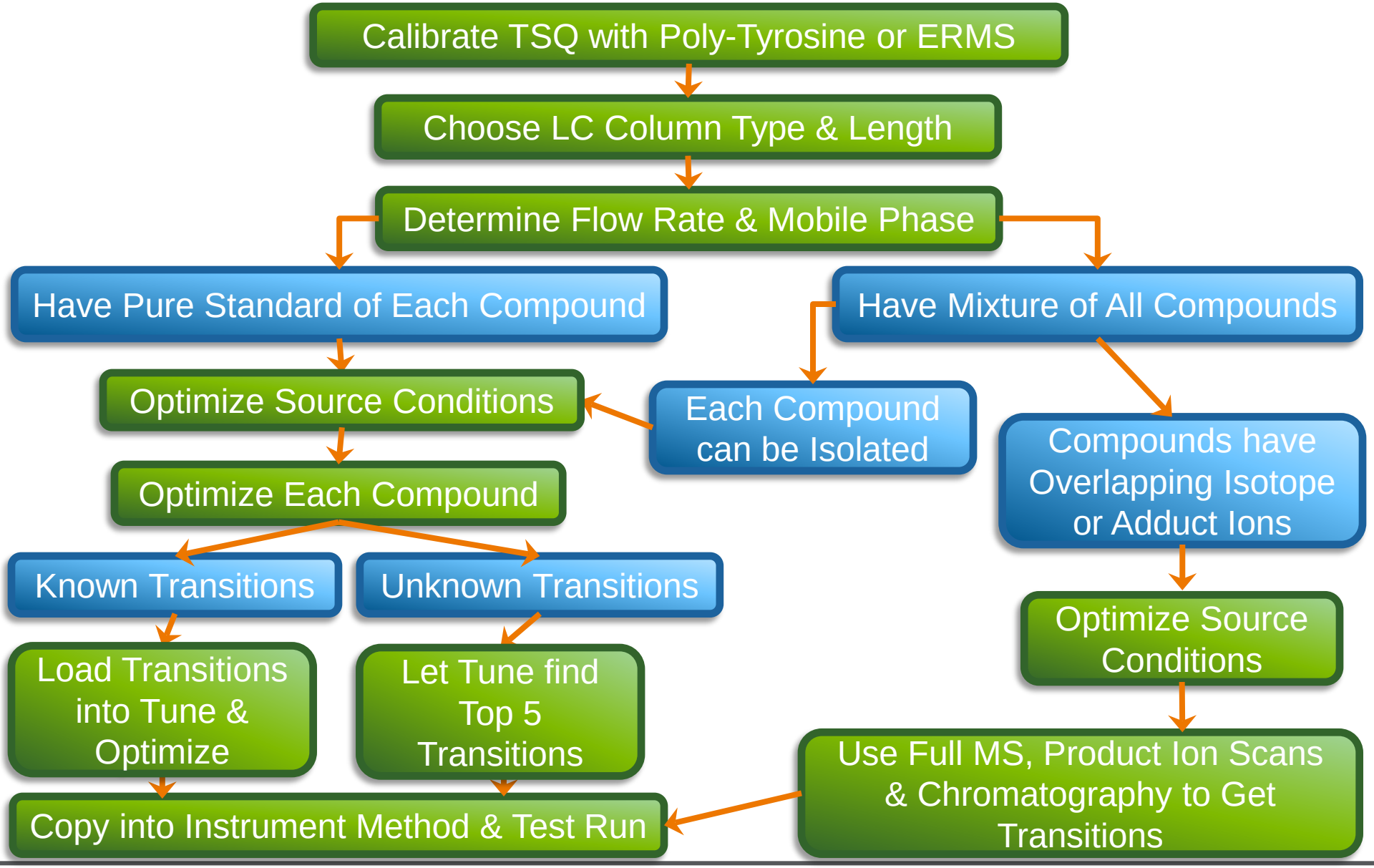
- Develop an instrument method for the separation of the Restek 525.2 Organonitrogen pesticide reference sample (Restek part number 33012) as a teaching example
- Understand the processes of *tuning*, *calibration*, *optimization*, and *method building*
- Explain which parameters in the method can be changed to increase data quality
- Analyze the resulting chromatography and data to evaluate whether further changes could help

Components

Alachlor (15972-60-8)
Ametryn (834-12-8)
Atraton (1610-17-9)
Atrazine (1912-24-9)
Bromacil (314-40-9)
Butachlor (23184-66-9)
Butylate (2008-41-5)
Chlorpropham (101-21-3)
Cyanazine (Bladex) (21725-46-2)
Cycloate (1134-23-2)
Diphenamid (957-51-7)
EPTC (759-94-4)
Etridiazole (2593-15-9)
Fenarimol (60168-88-9)
Fluridone (Sonar) (59756-60-4)
Hexazinone (Velpar) (51235-04-2)
Metolachlor (51218-45-2)
Metribuzin (21087-64-9)

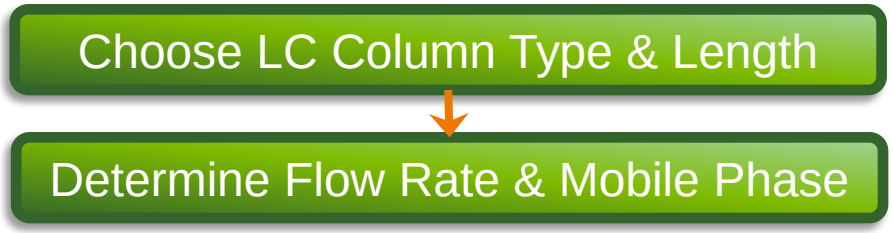
MGK-264 (113-48-4)
Molinate (2212-67-1)
Napropamide (Devrinol) (15299-99-7)
Norflurazon (27314-13-2)
Pebulate (1114-71-2)
Prometon (1610-18-0)
Prometryne (7287-19-6)
Propachlor (1918-16-7)
Propazine (139-40-2)
Propyzamide (23950-58-5)
Simazine (122-34-9)
Simetryn (1014-70-6)
Tebuthiuron (34014-18-1)
Terbacil (5902-51-2)
Terbutryn (886-50-0)
Triadimefon (43121-43-3)
Tricyclazole (Beam) (41814-78-2)
Trifluralin (1582-09-8)
Vernolate (1929-77-7)

Process Flow Chart

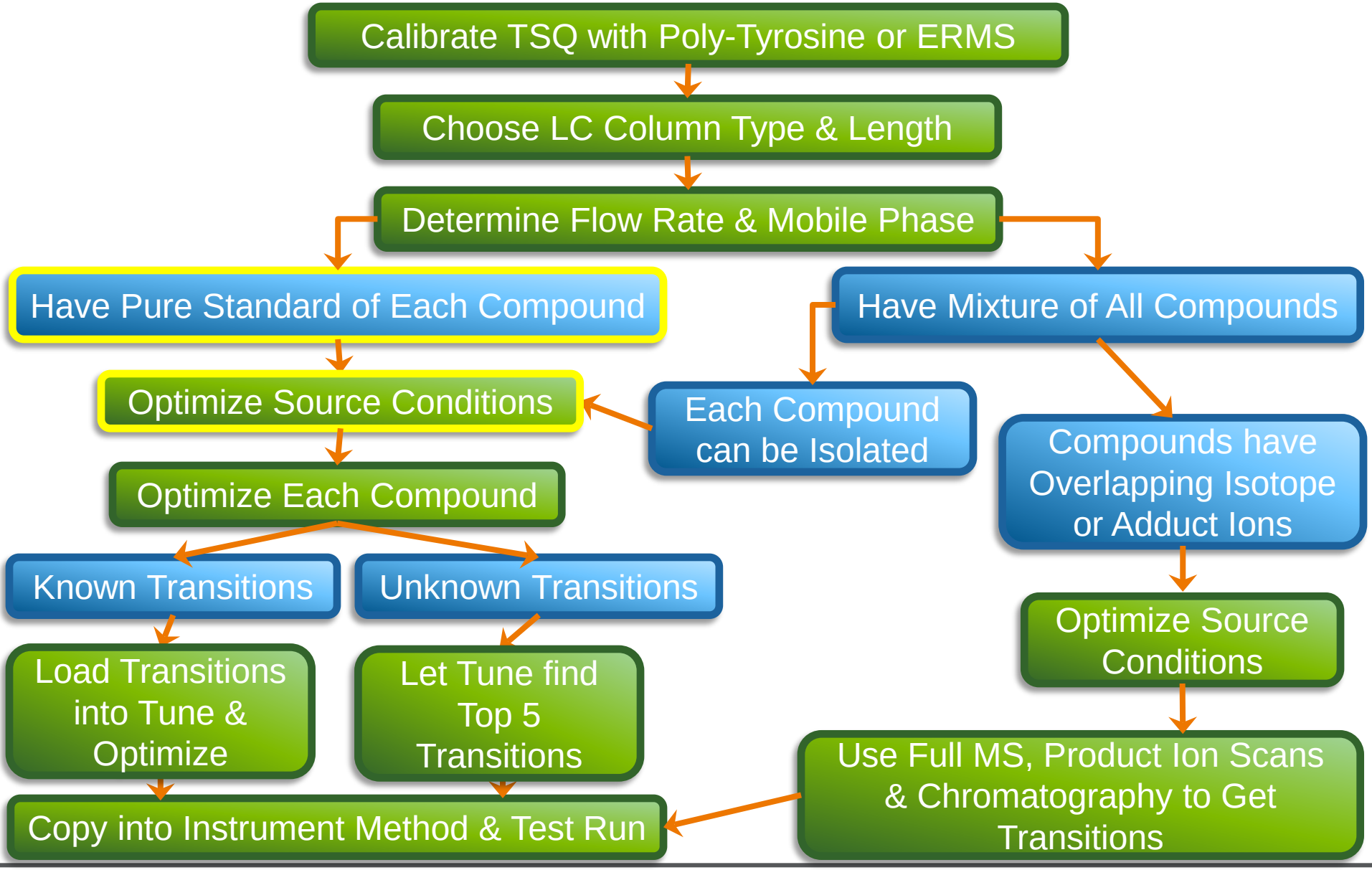


Choosing Chromatography Method

- This method was chosen to produce that fastest possible separation of the 37 component Restek 525.2 organonitrogen pesticide mix
- Ultimate 3000 RSLC with a HPG pump and a temperature controlled column compartment
- Accucore™ Vanquish™ C_{18}^{+} 2.1 mm x 50 mm, 1.5 um (PN 27101-052130) HPLC column running at 400 uL/min
- Gradient:
  - Mobile Phase A: 0.1% Formic Acid in Water (PN LS118-1)
  - Mobile Phase B: 0.1% Formic Acid in Acetonitrile (PN LS120-1)
  - 1 minute gradient form 10% B to 90% B
  - 3.5 minute total run time



Optimizing Source Conditions When You Have Pure Compounds

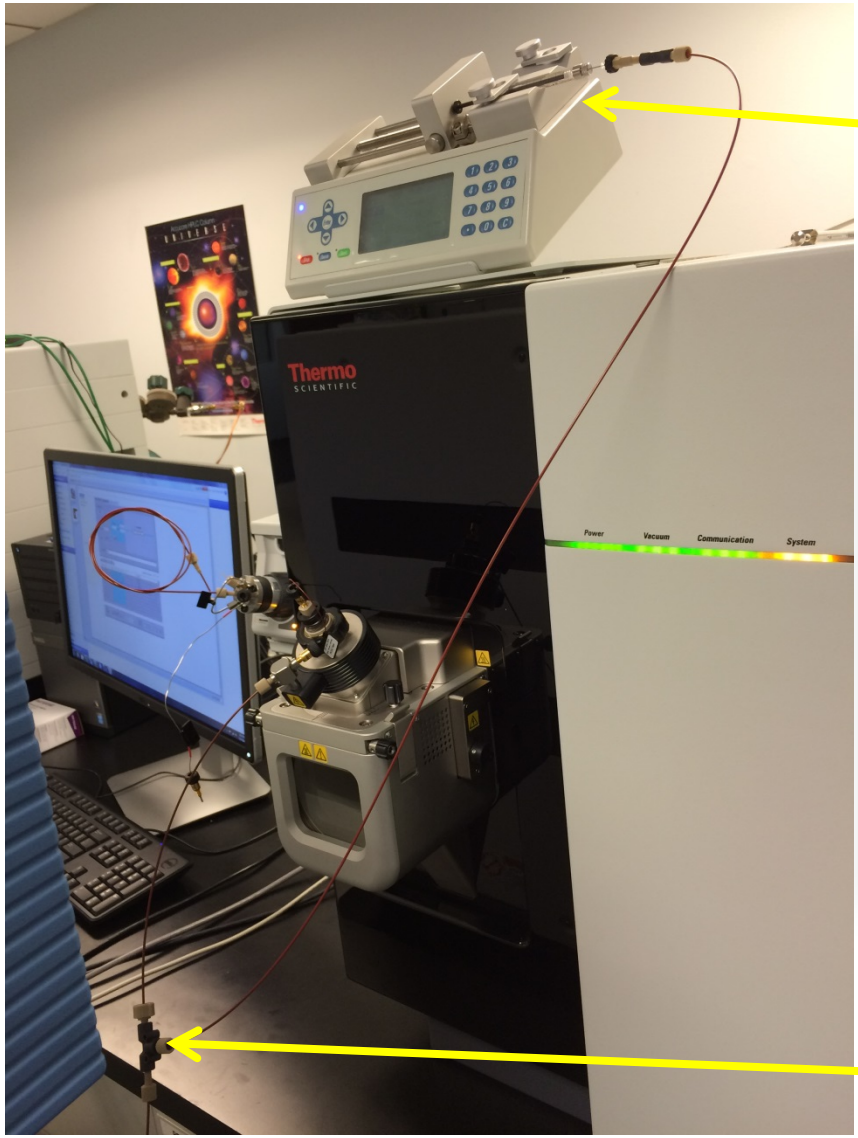


Steps to Start Up

- Place the mass spectrometer into operate mode
- Tee into the LC flow with your compound of interest (1 ng/uL is good starting level)
- Set the gases / temperatures using the ‘Get Defaults’
- Connect the tee outlet into the mass spectrometer

Optimize Source Conditions

Compounds Teed in for Optimization



Compound in Syringe Pump

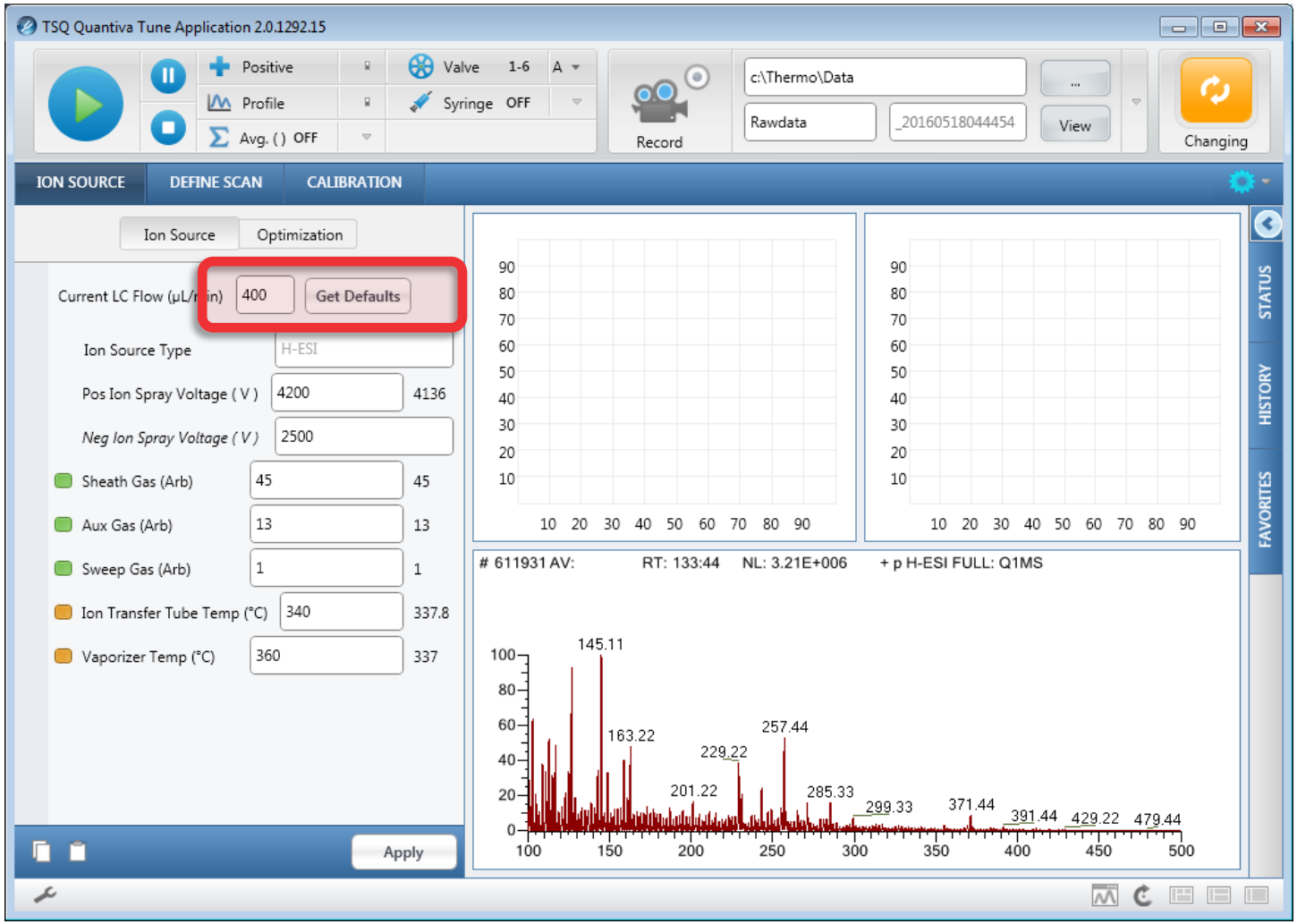
- Part Numbers (www.idex-hs.com):
  - F-130: Fingertight fittings, PEEK
  - P-727: Tee, PEEK
  - 1535: Red PEEK Tubing, 0.005” ID
  - 9013: Syringe Adaptor (Blunt syringe)

    or
  - P-642: Luer Adaptor (Luer syringe)

Tee connected to LC output

Use ‘Get Defaults’ to Set Basic Instrument Parameters

TSQ Tune Page



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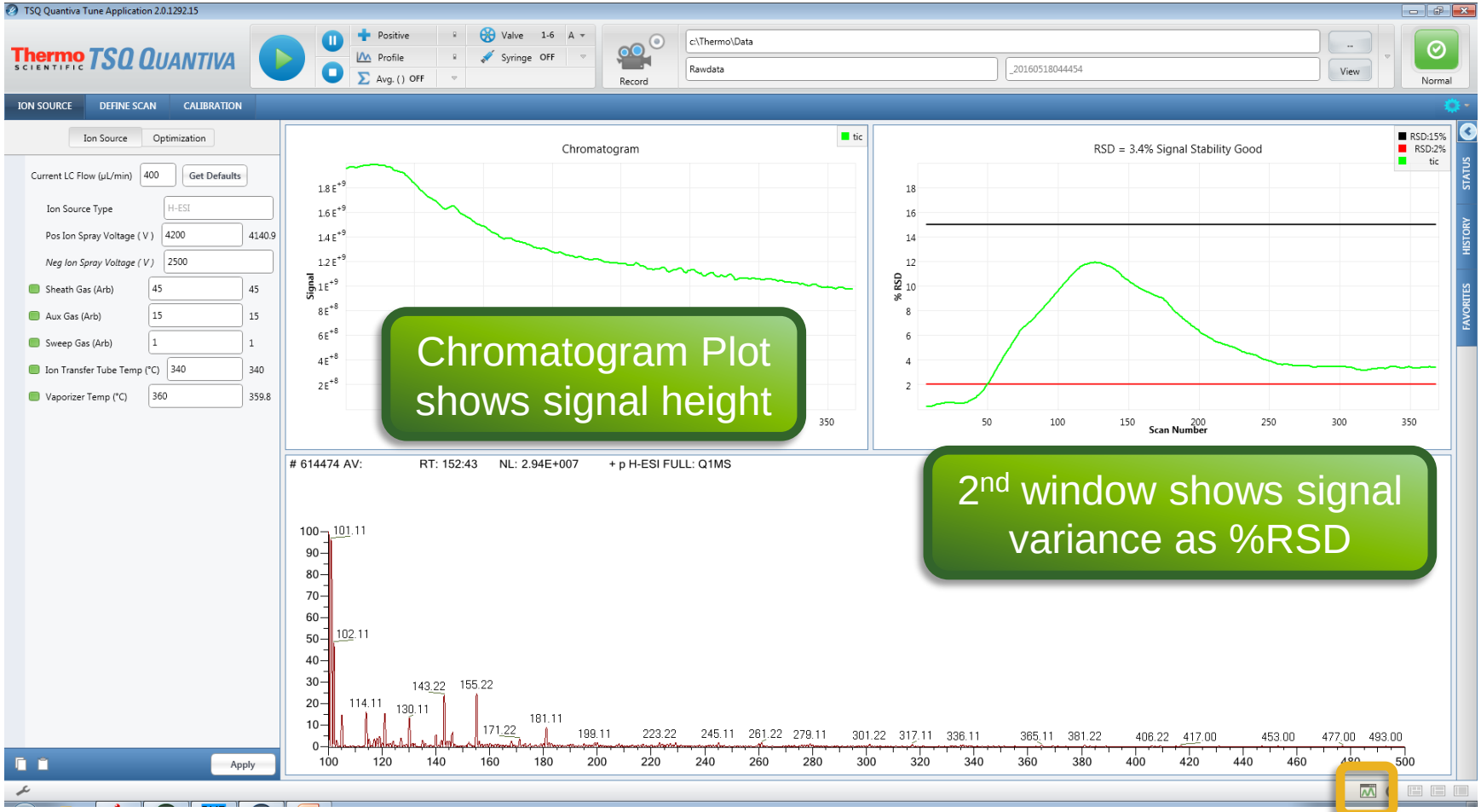
Tune Window with LC Flow Teed into the Syringe Pump

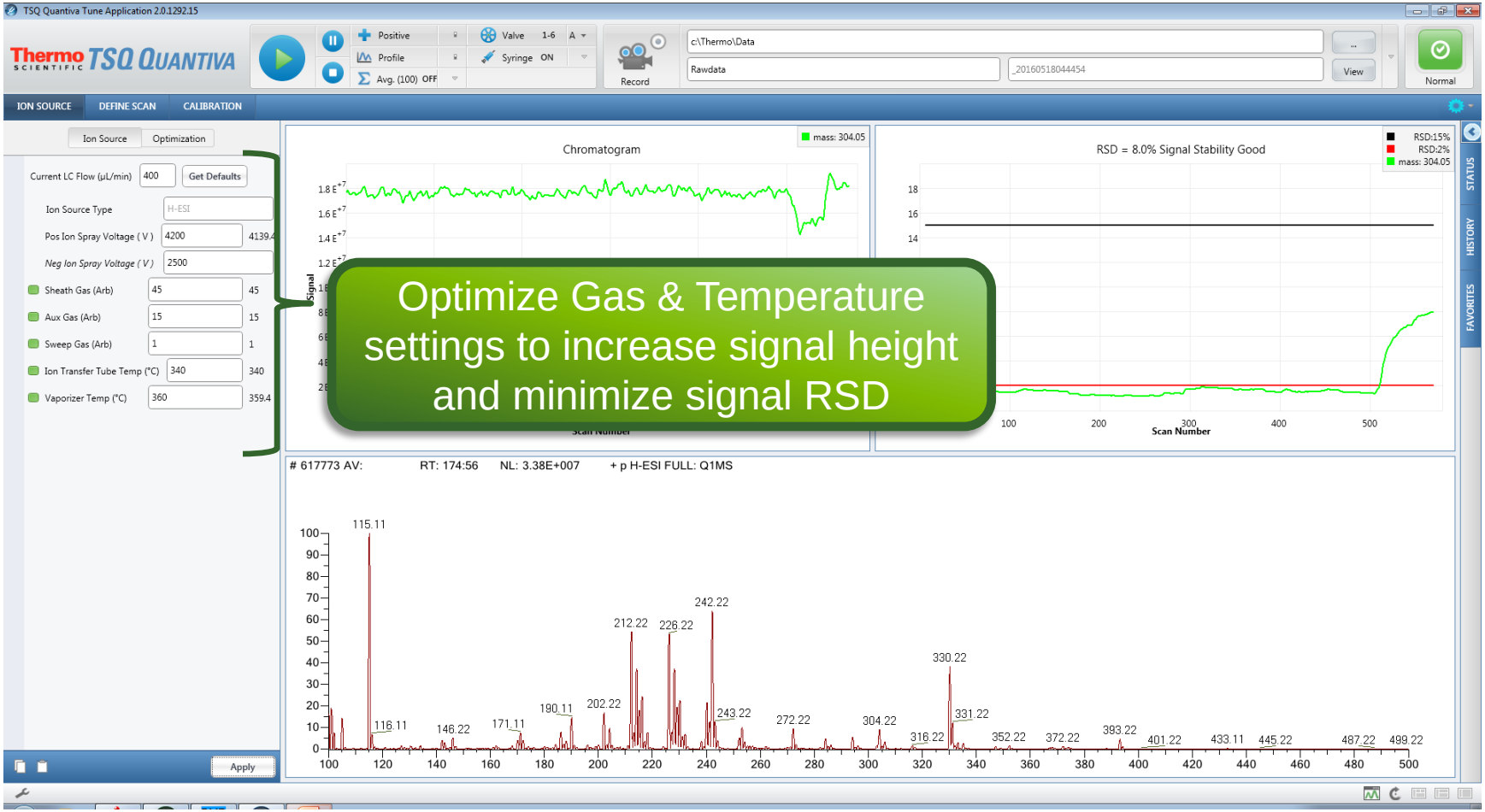


Parameters to Optimize

- One compound is used to optimize *ion source conditions* to make the most stable spray:
  - Probe Position
  - Sheath gas
  - Aux gas
  - Sweep gas
  - Spray Voltage
  - Source Temperatures

- Each compound is used to optimize the *mass spectrometer parameters* for the best possible response and selectivity:
  - Precursor m/z
  - RF-Lens voltage
  - Product ions / SRM transitions
  - Collision Energies
  - Ion Polarity

Optimization of Default Source Conditions Manually



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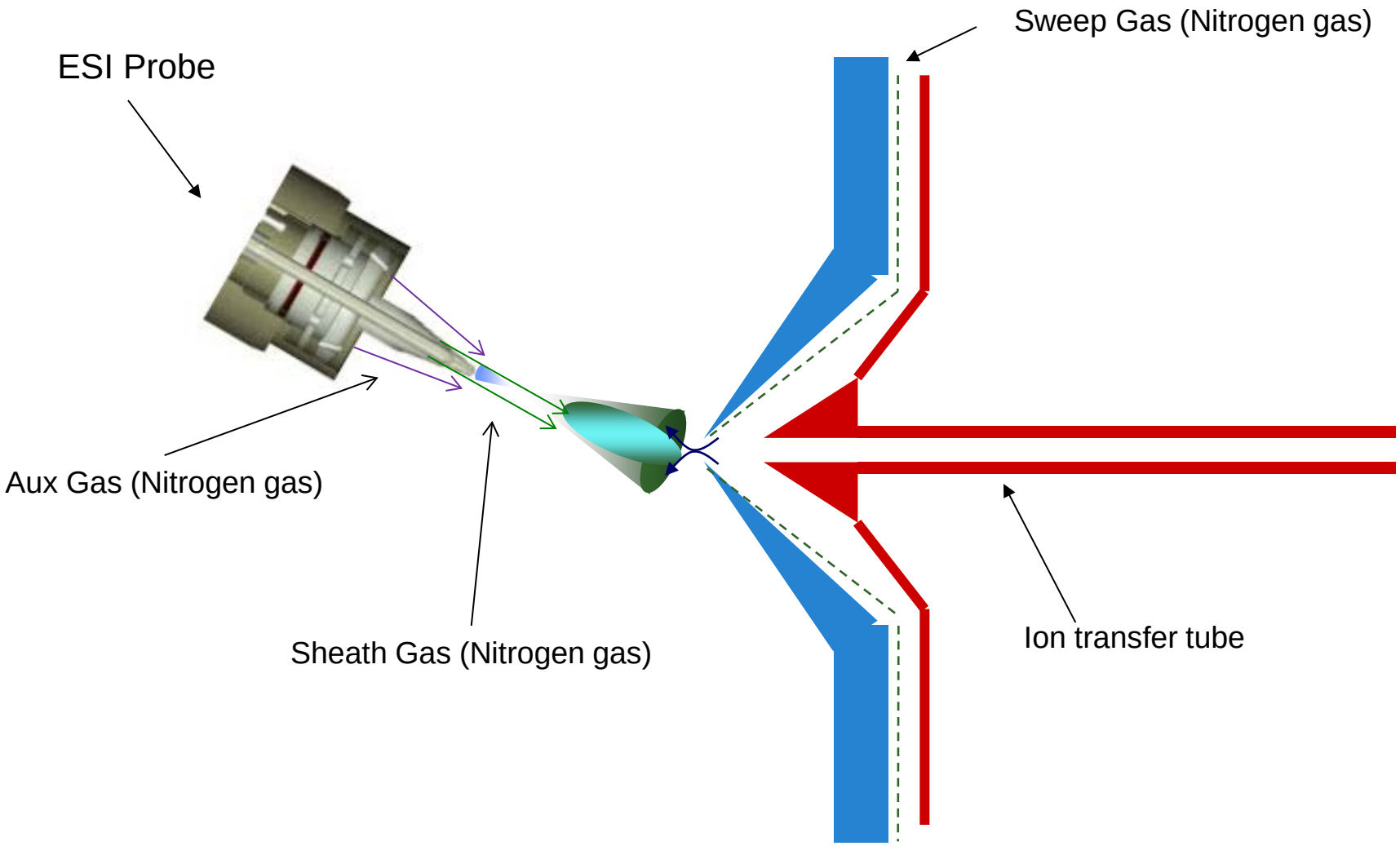
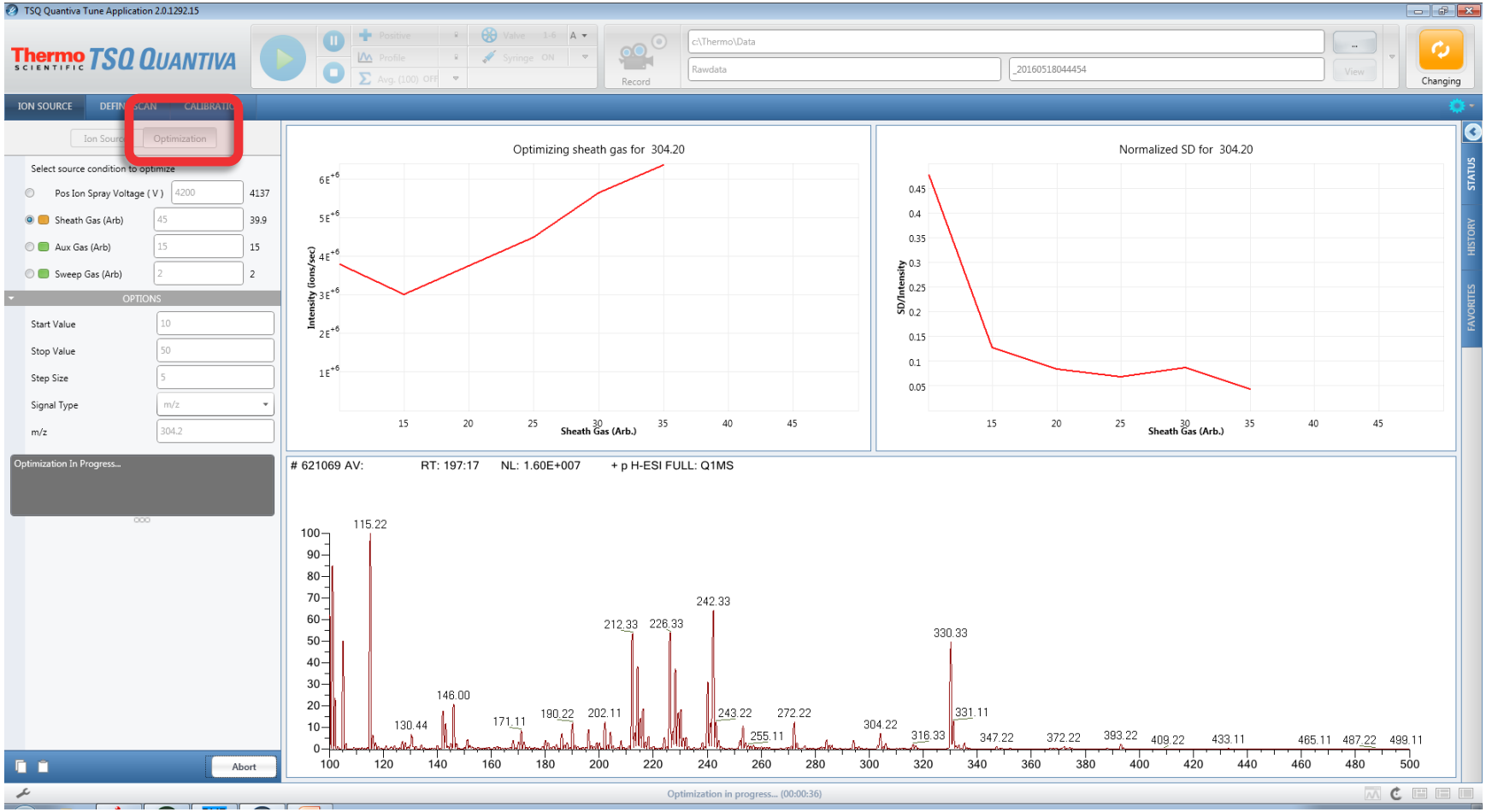
Probe Gases



Optimization of Source Conditions Using Automated Routines



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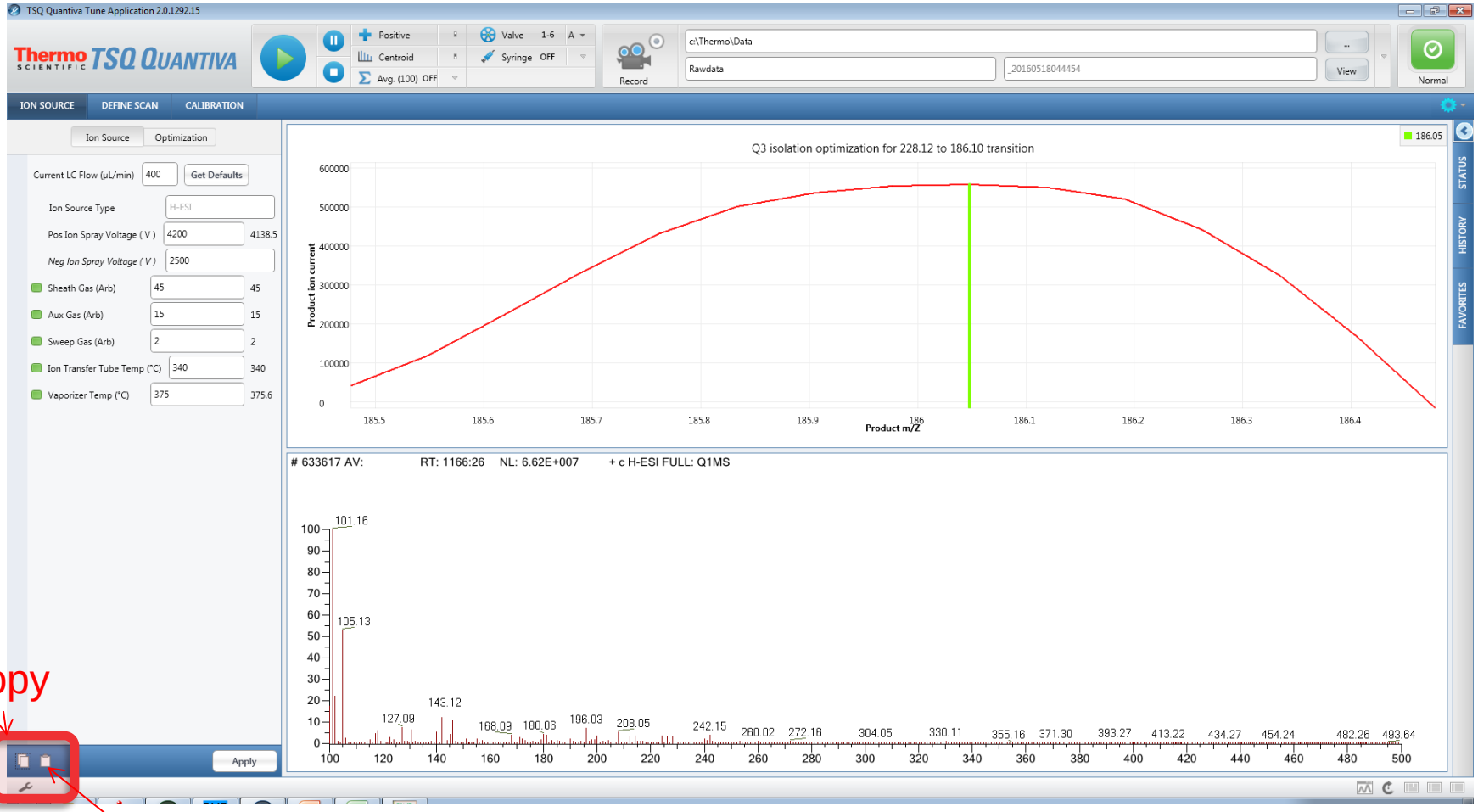
Copy Source Conditions

TSQ Tune Page



Copy

Paste

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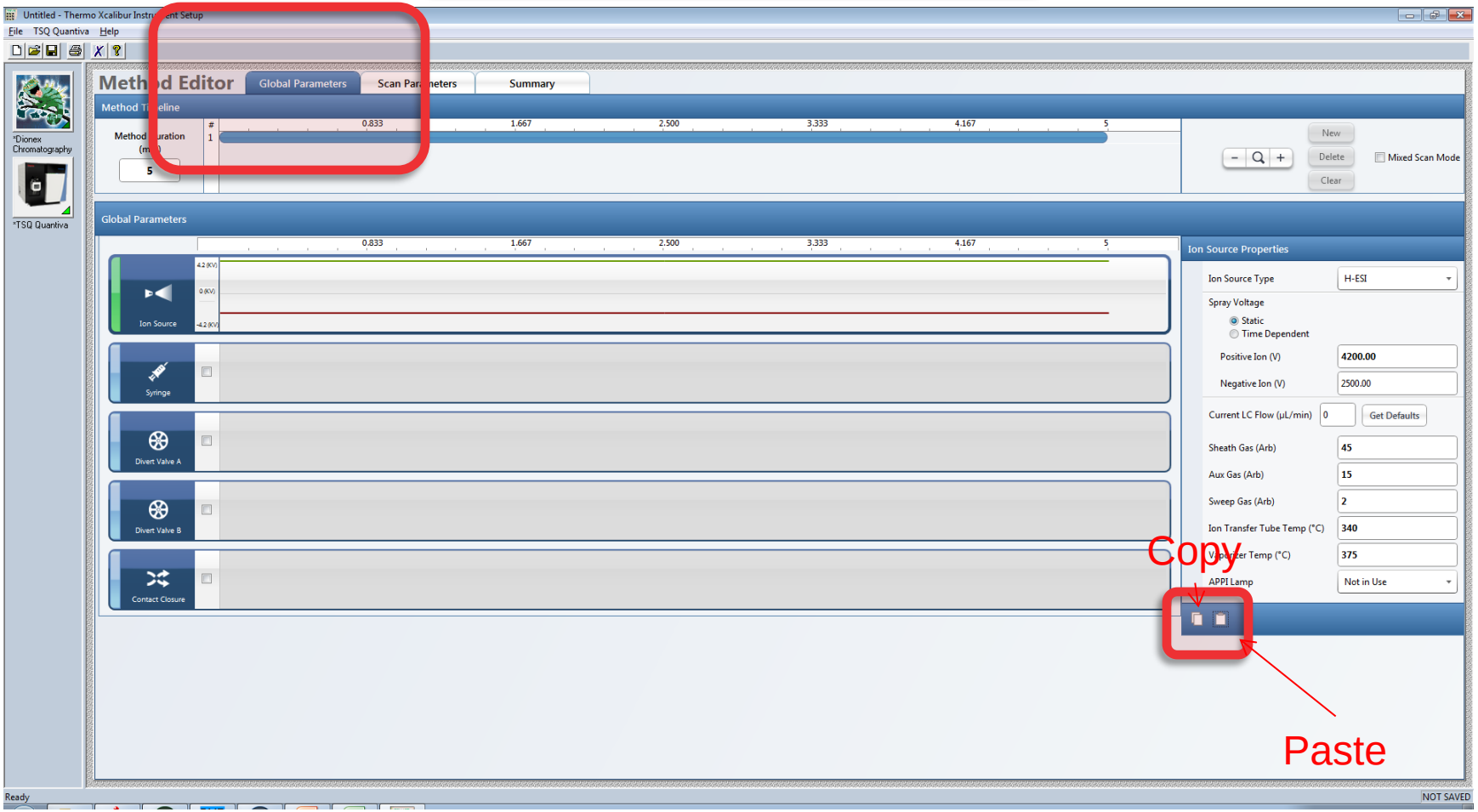
Paste Source Conditions

TSQ Instrument Method Editor



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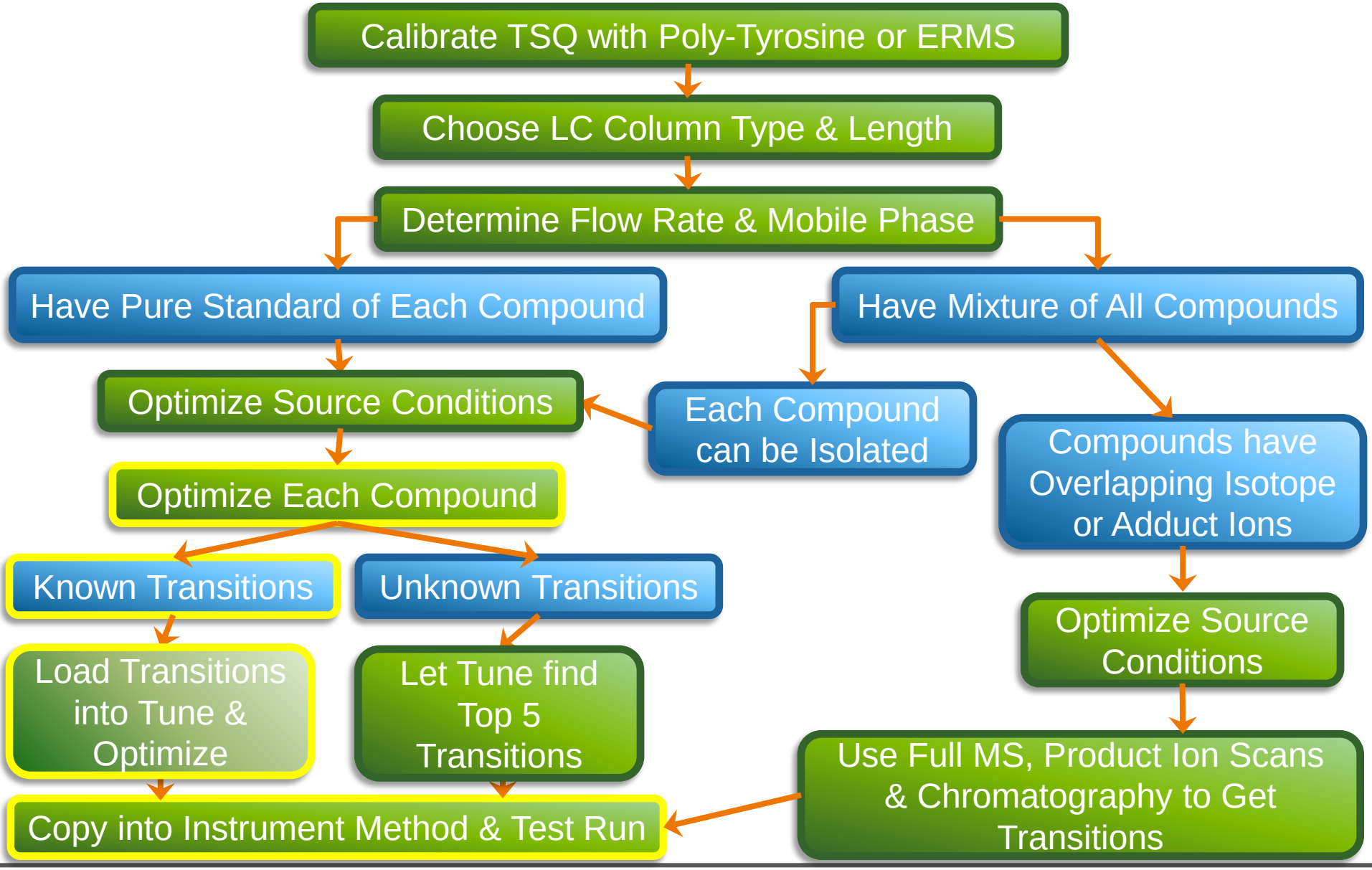
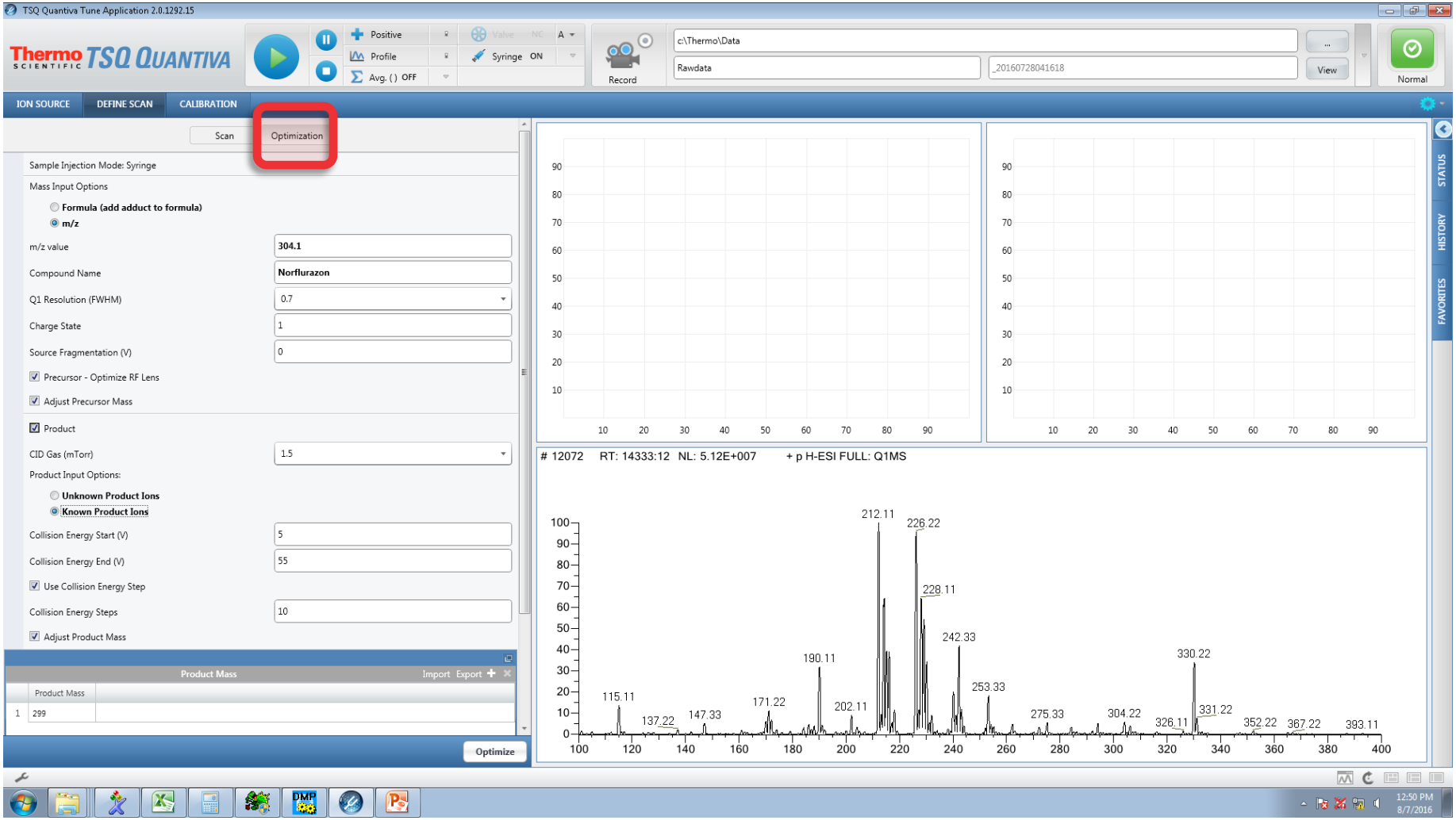
Compound Optimization When the Transitions Are Known



Compound Optimization of the Precursor



Optimize Each Compound

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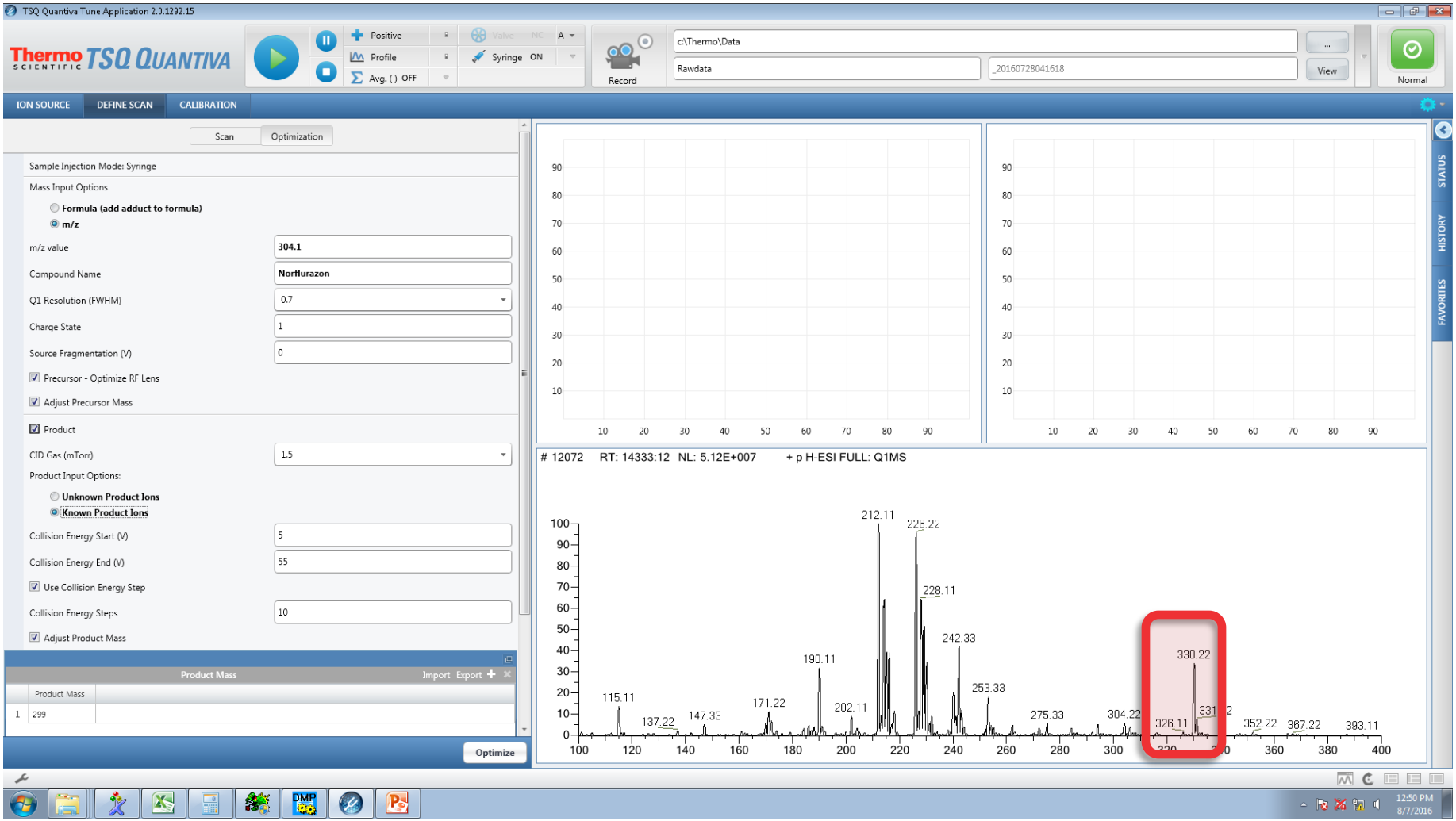
Which Precursor Do We Use?



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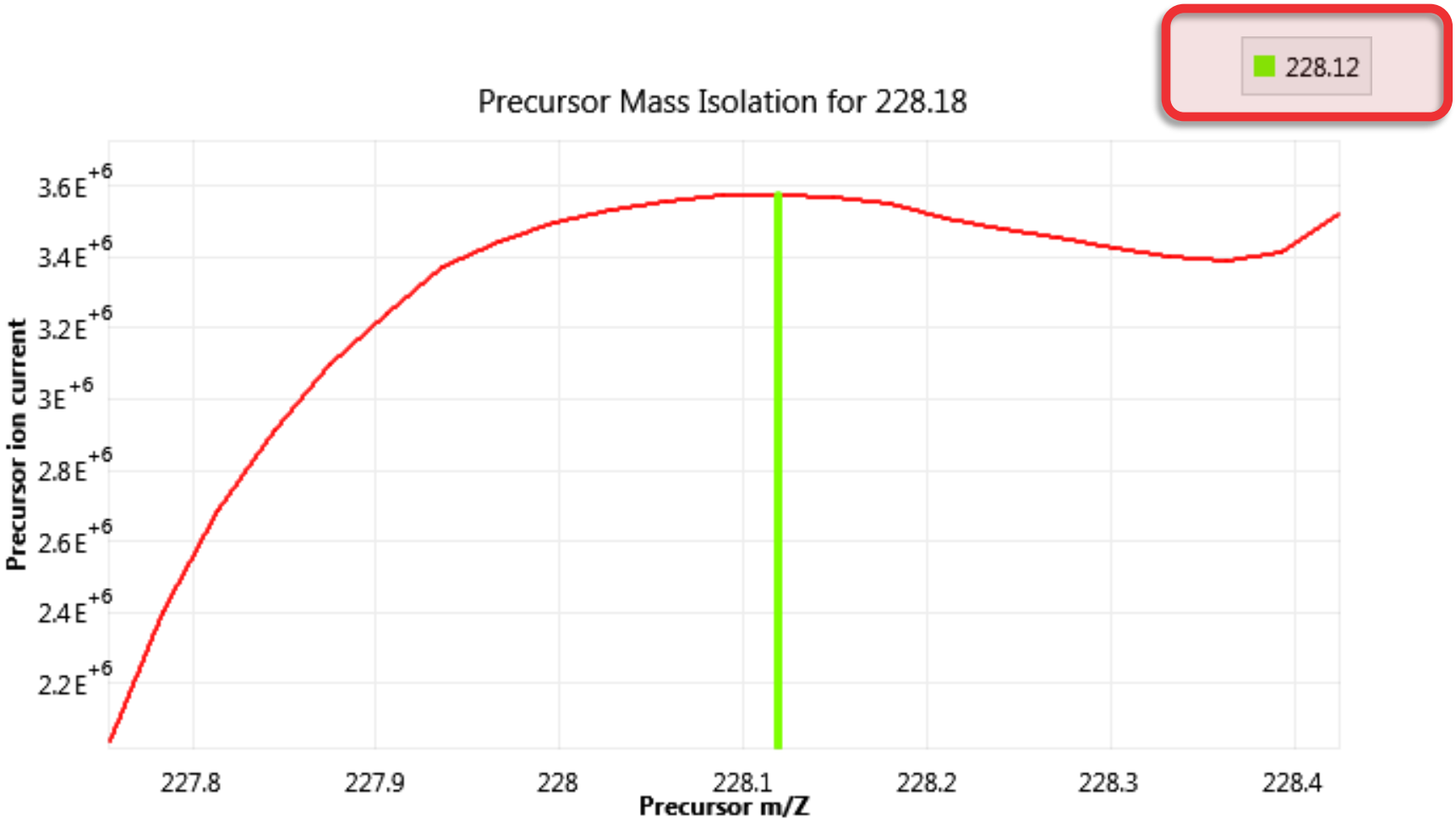
Optimizes Precursor m/z



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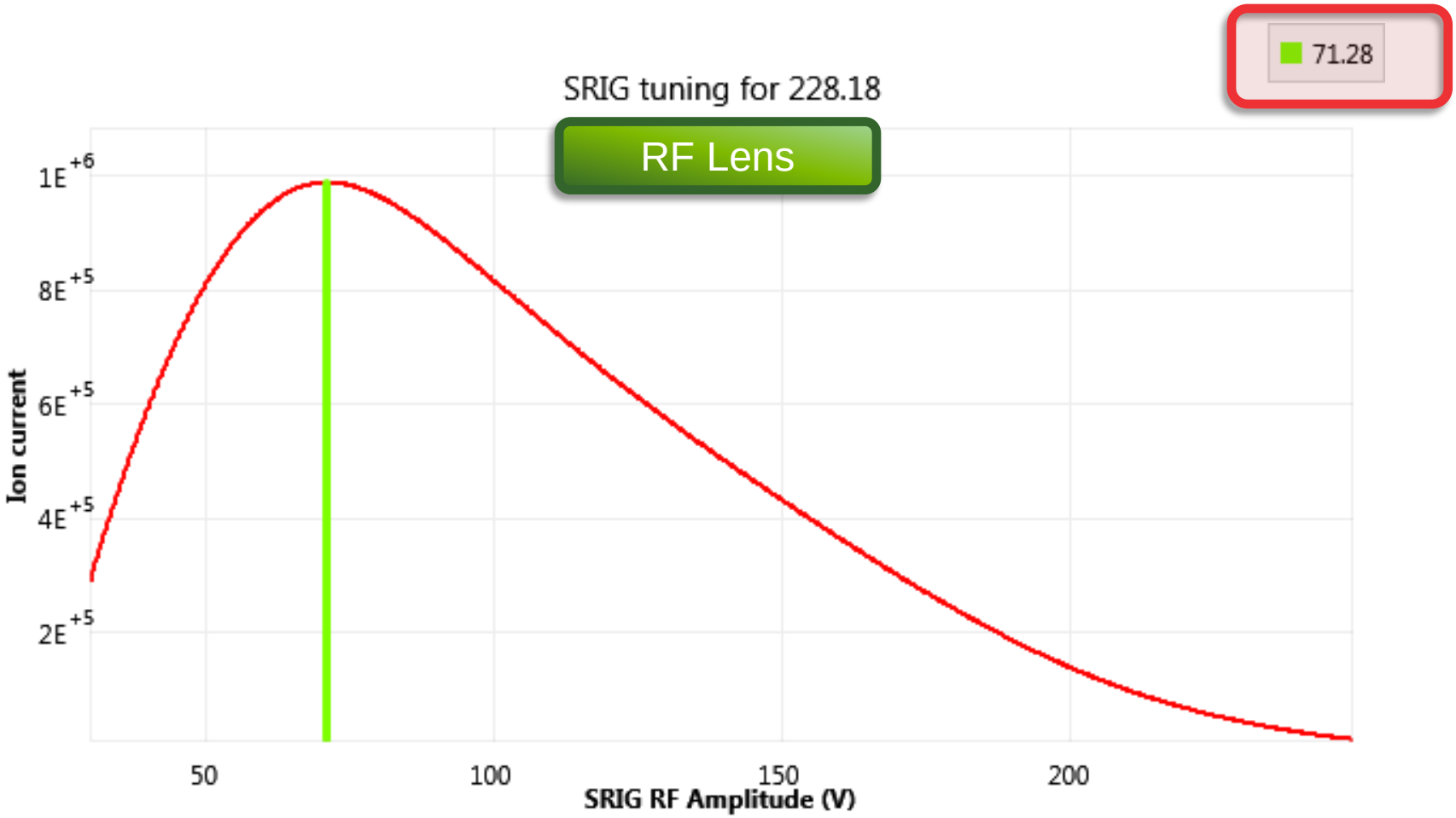
Optimizes RF Lens



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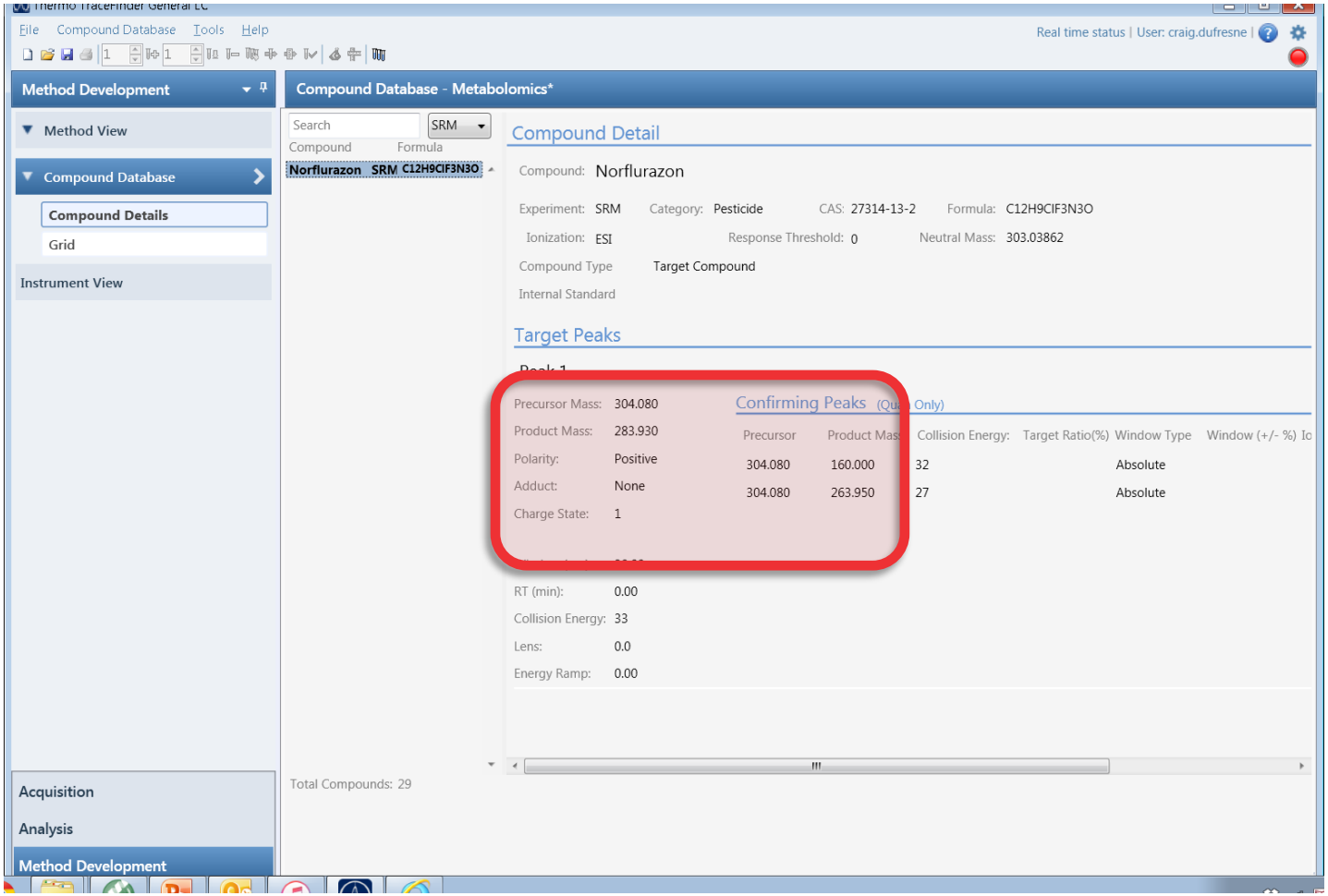
Using Product Ions from a TraceFinder Compound Database



Known Transitions

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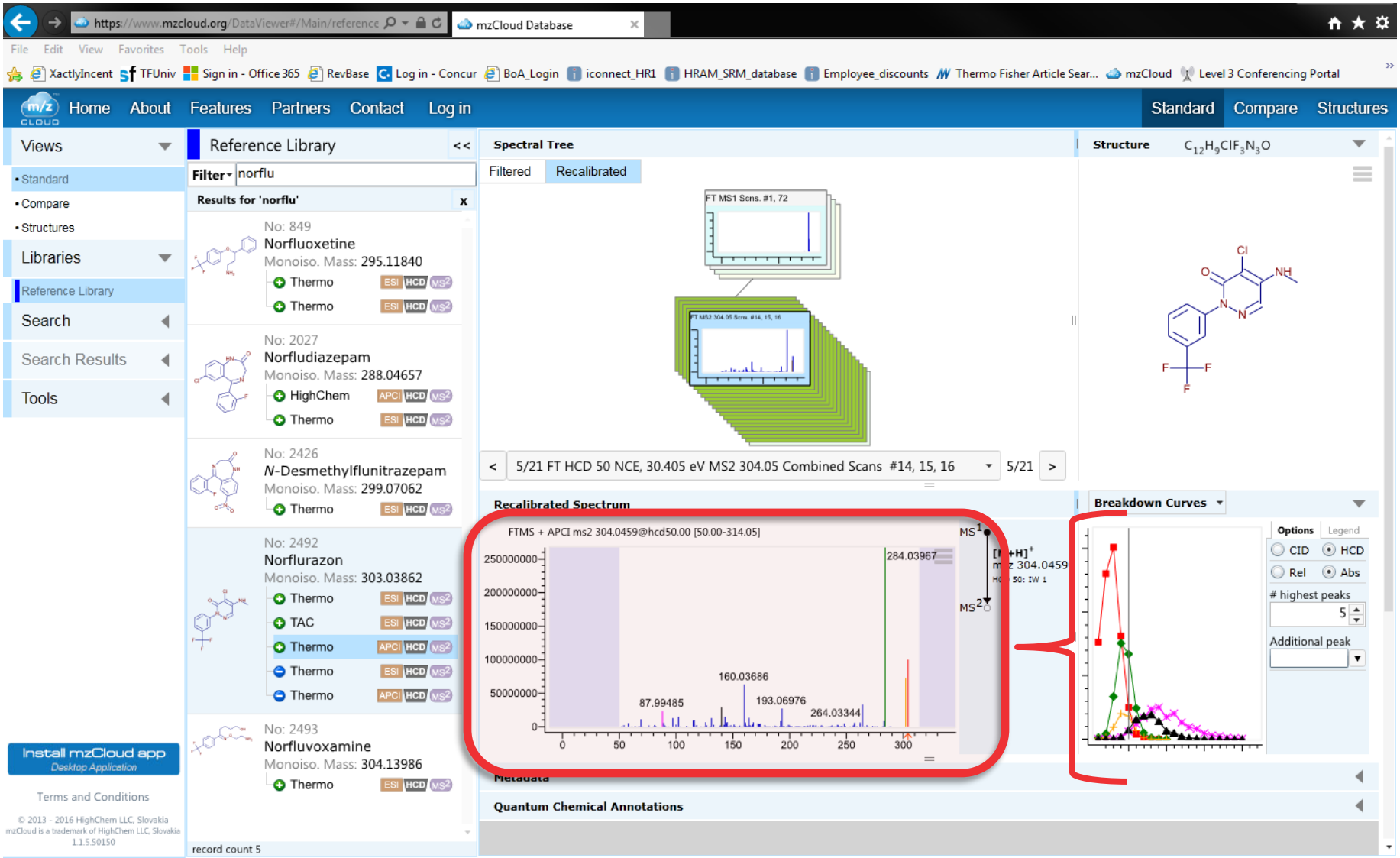
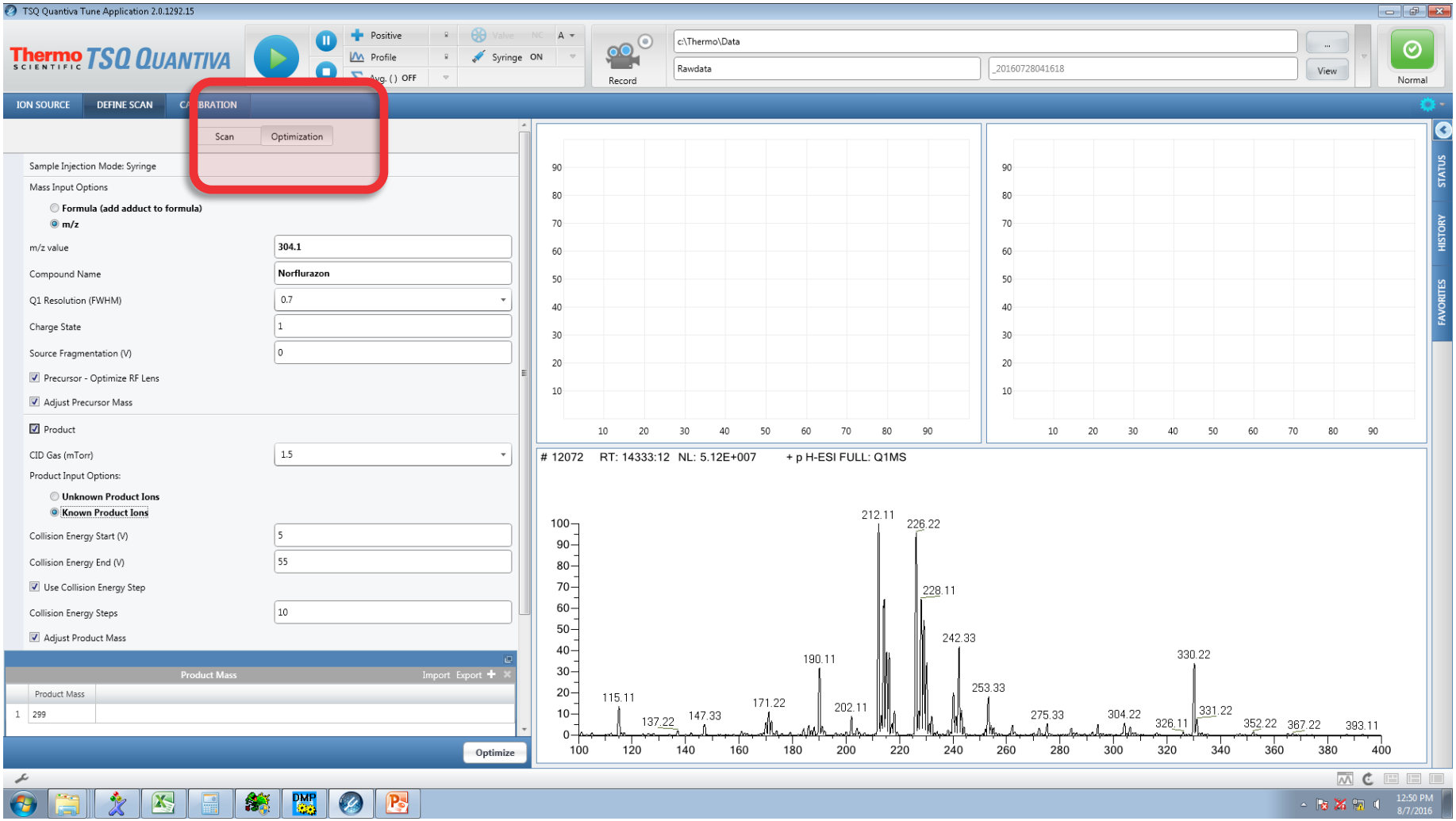
Choosing Transitions from mzcloud.org



Compound Optimization of Known Product Ions



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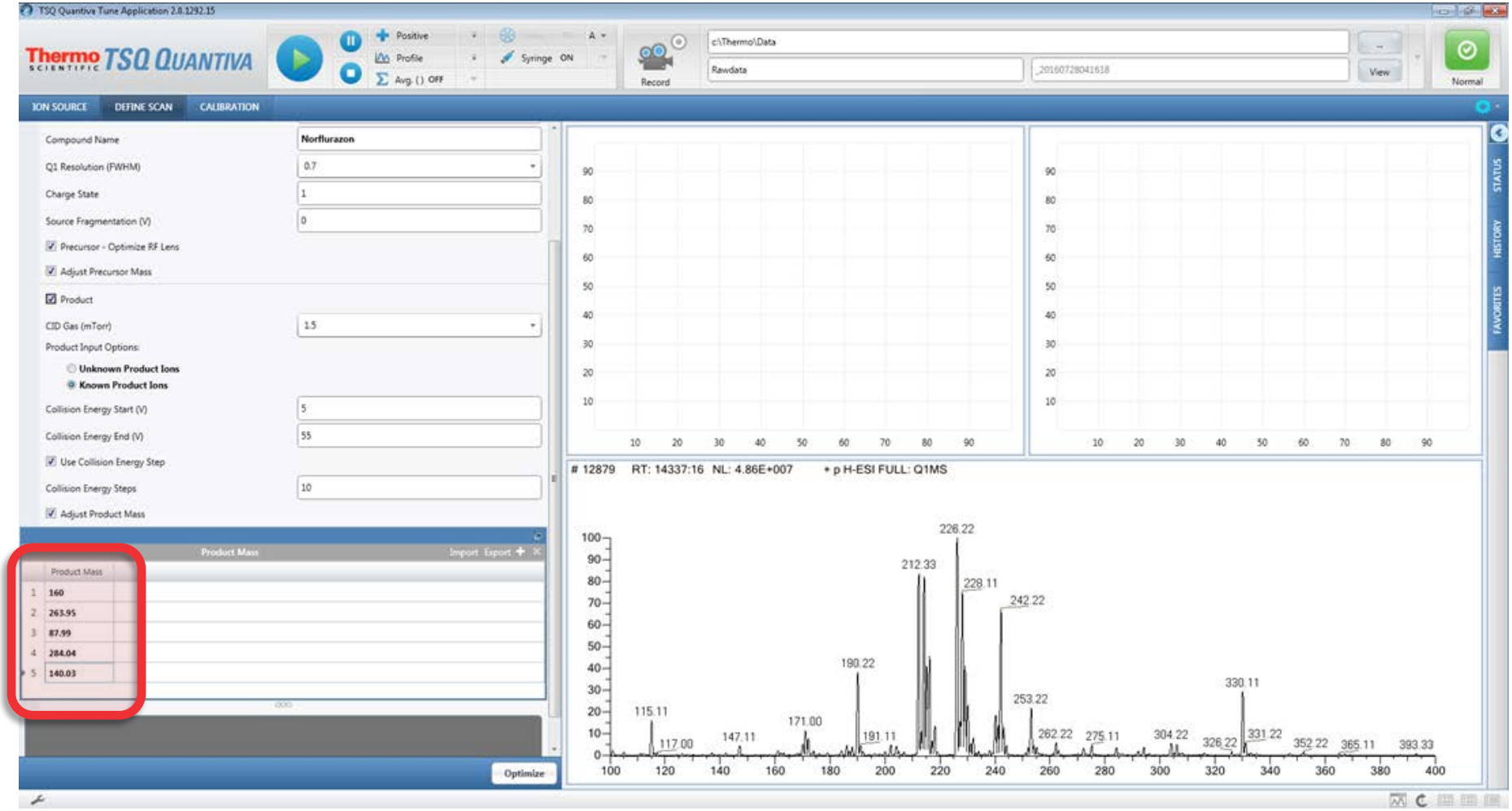
Typing in the Known Product Ions



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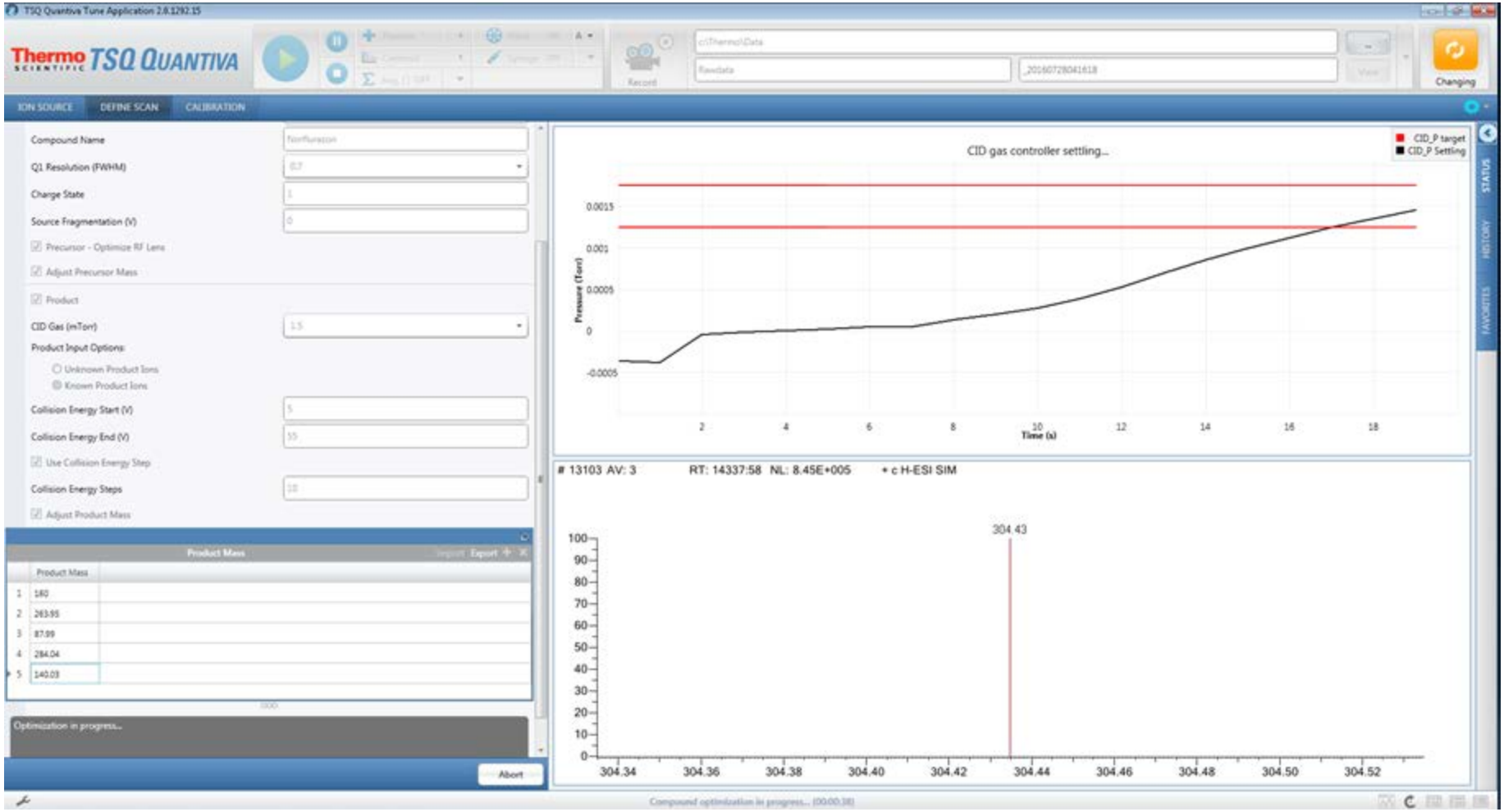
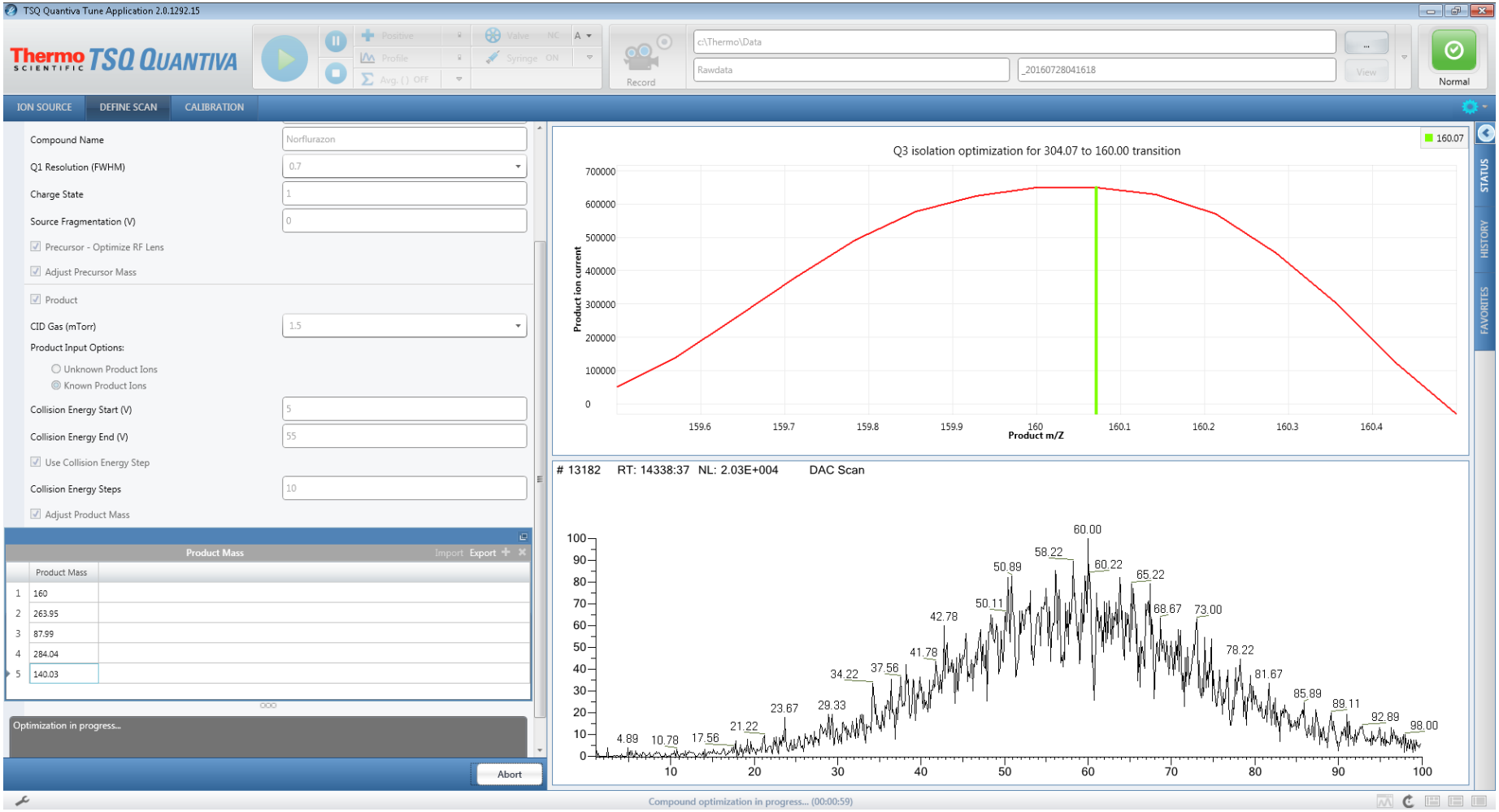
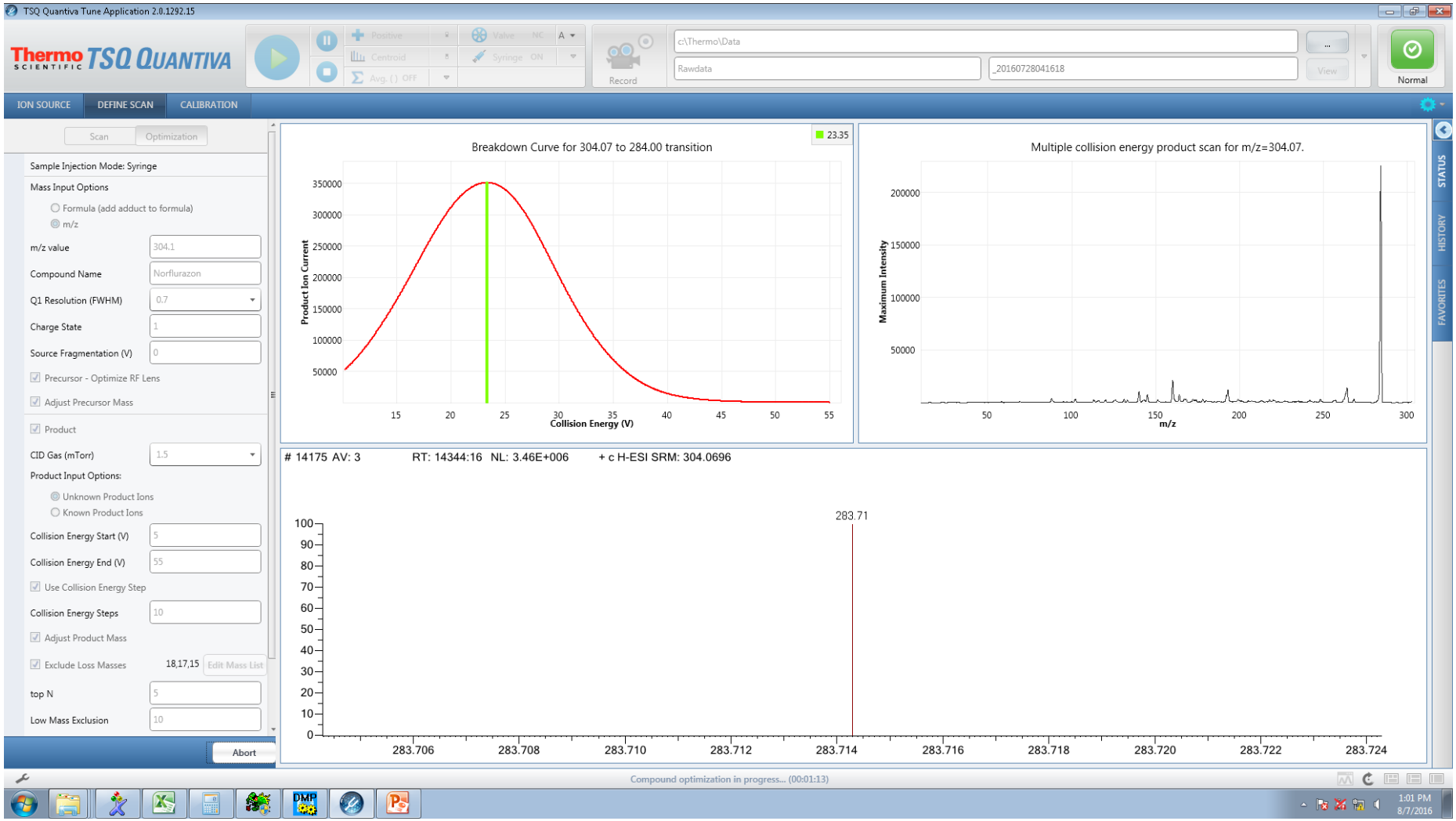
The Instrument Stabilizes the Collision gas in Q2



The Product Ions are Optimized



Breakdown Curves



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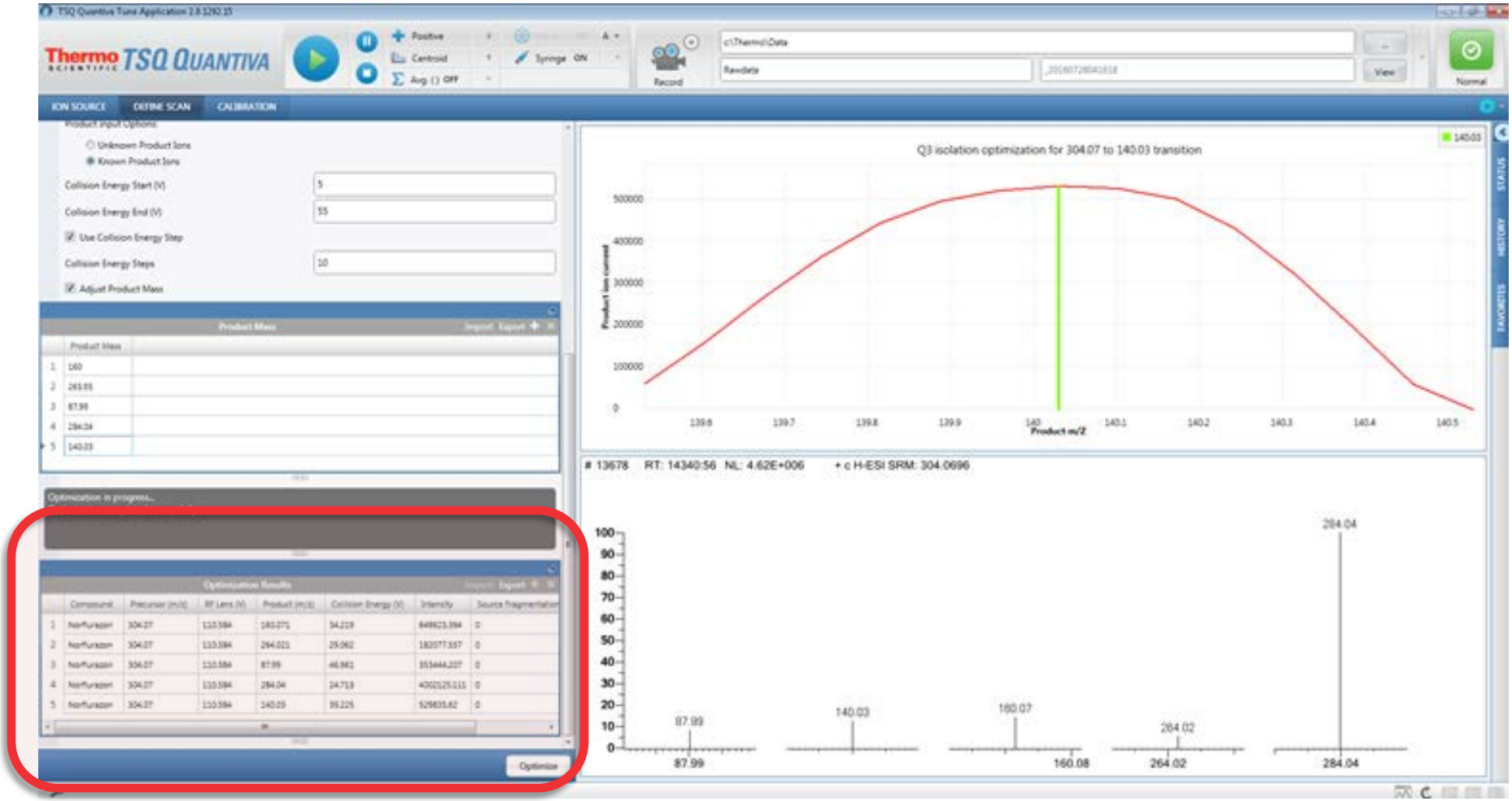
Copying the Transitions to the Instrument Method



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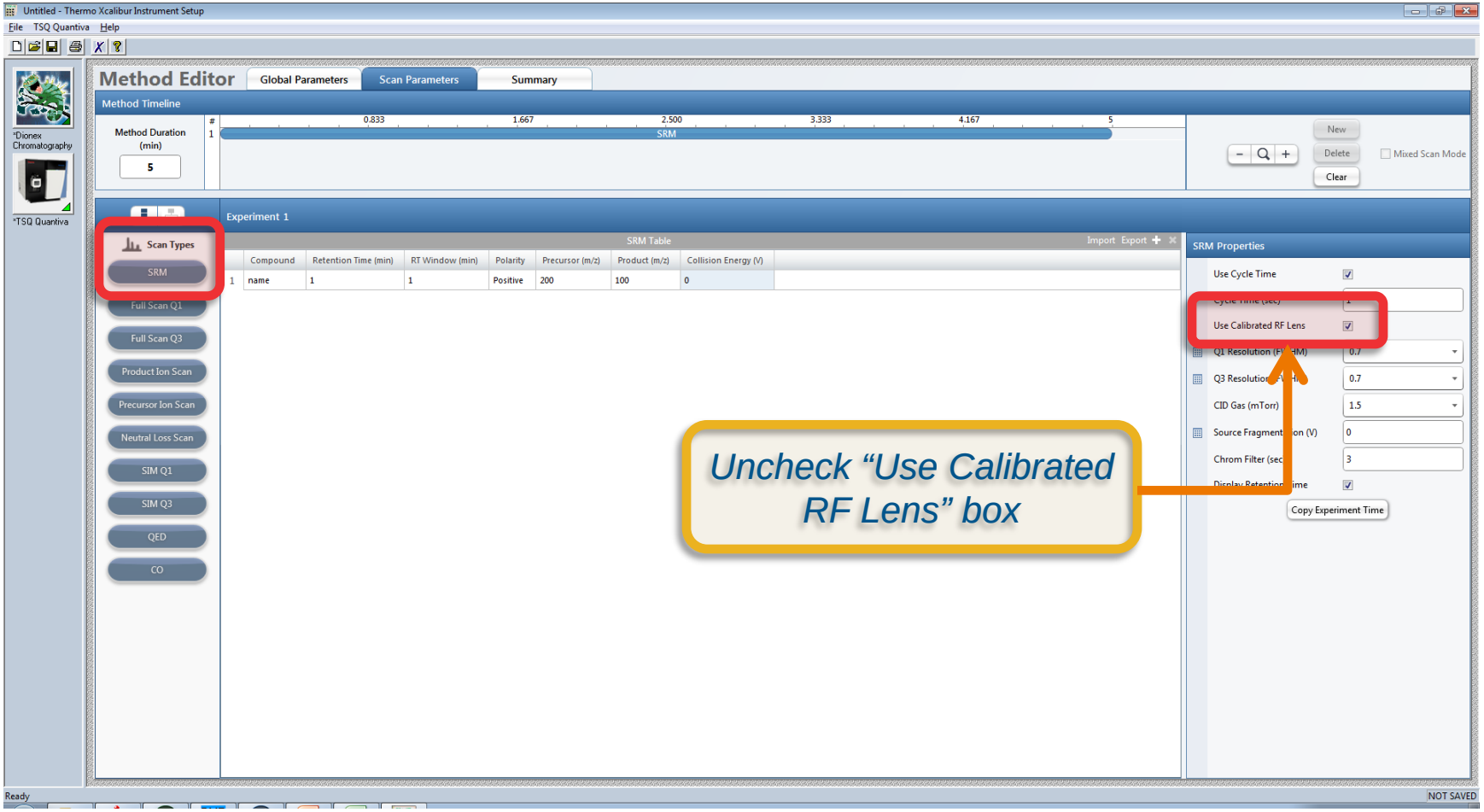
Drag and Drop SRM Scan

TSQ Instrument Method Editor



Uncheck "Use Calibrated RF Lens" box

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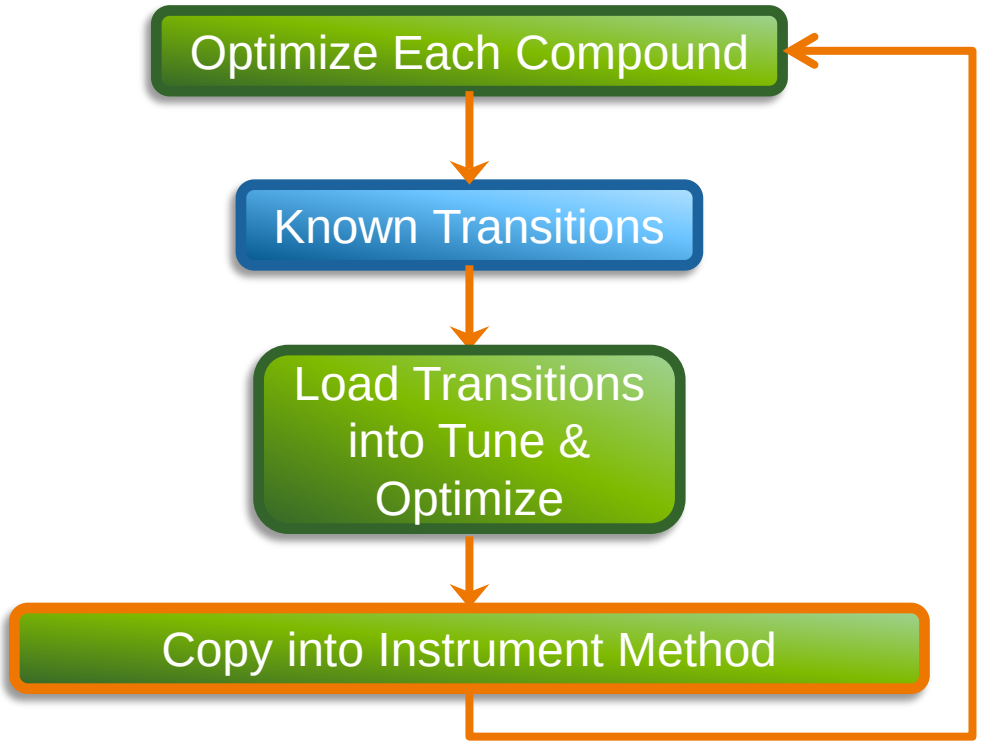
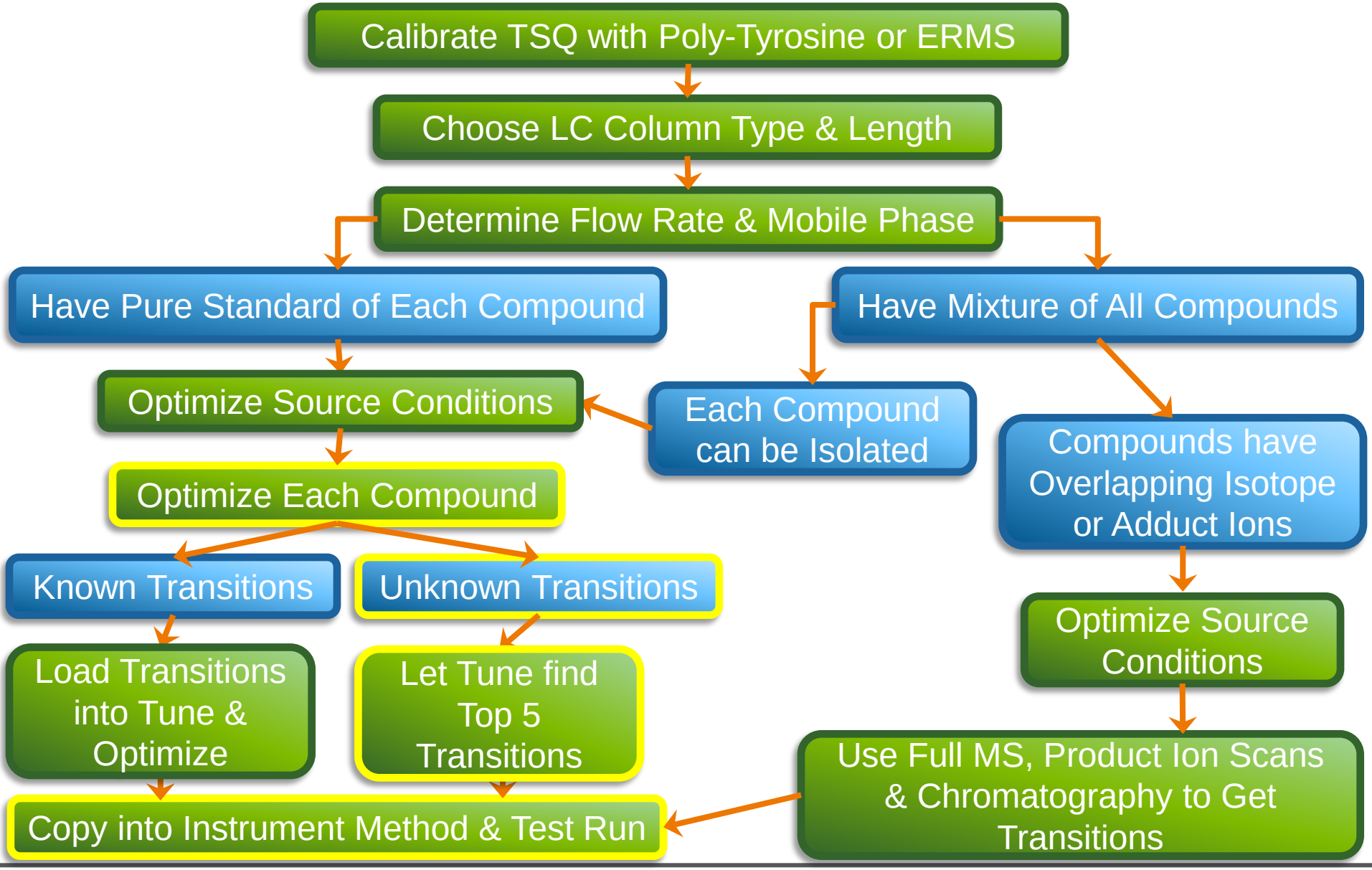
Repeat for Each Compound



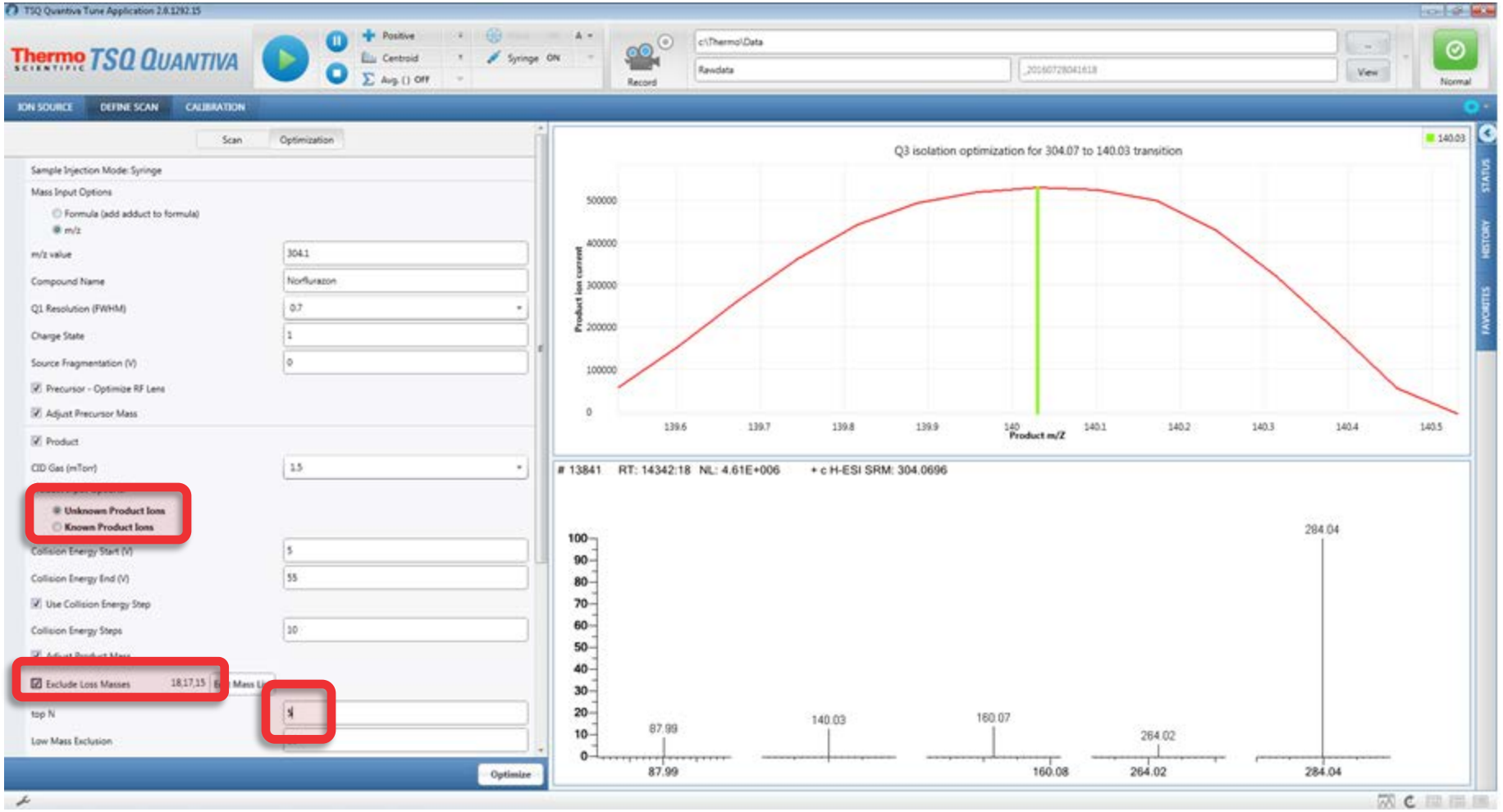
Compound Optimization When the Transitions Aren't Known



Thoughts on Choosing Unknown Product Ions…

- Try to avoid choosing common losses such as ammonia and water as these add little specificity to the SRM transition
- Be aware of using high collision energies and generating fragment ions that are very small and common to a large number of compounds
- In general its desirable to choose five product ions:
  - One QUAN ION for measuring peak area for your curve
  - Two CONFIRMING IONS to use for ion ratio measurements to validate proper peak picking during complex matrix quantitation
  - Two back up ions to use in case of matrix interference requiring the removal of a quan or confirming ion
- Mass Frontier is an excellent package for theoretically fragmenting a ion and evaluating its potential product ions

Setting Up Compound Optimization to Find Unknown Transitions



Unknown Transitions

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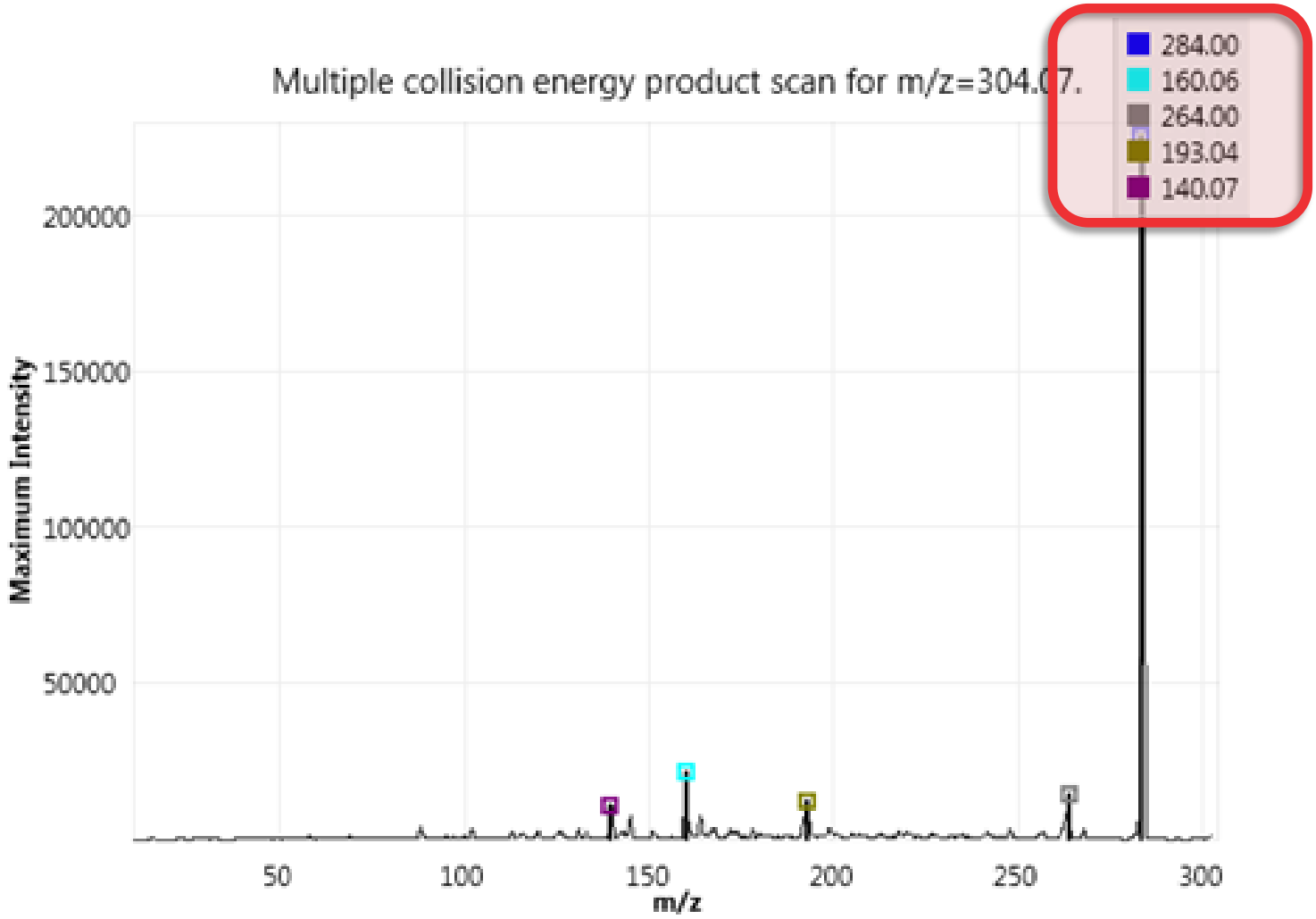
The Instrument Finds the Best Product Ions



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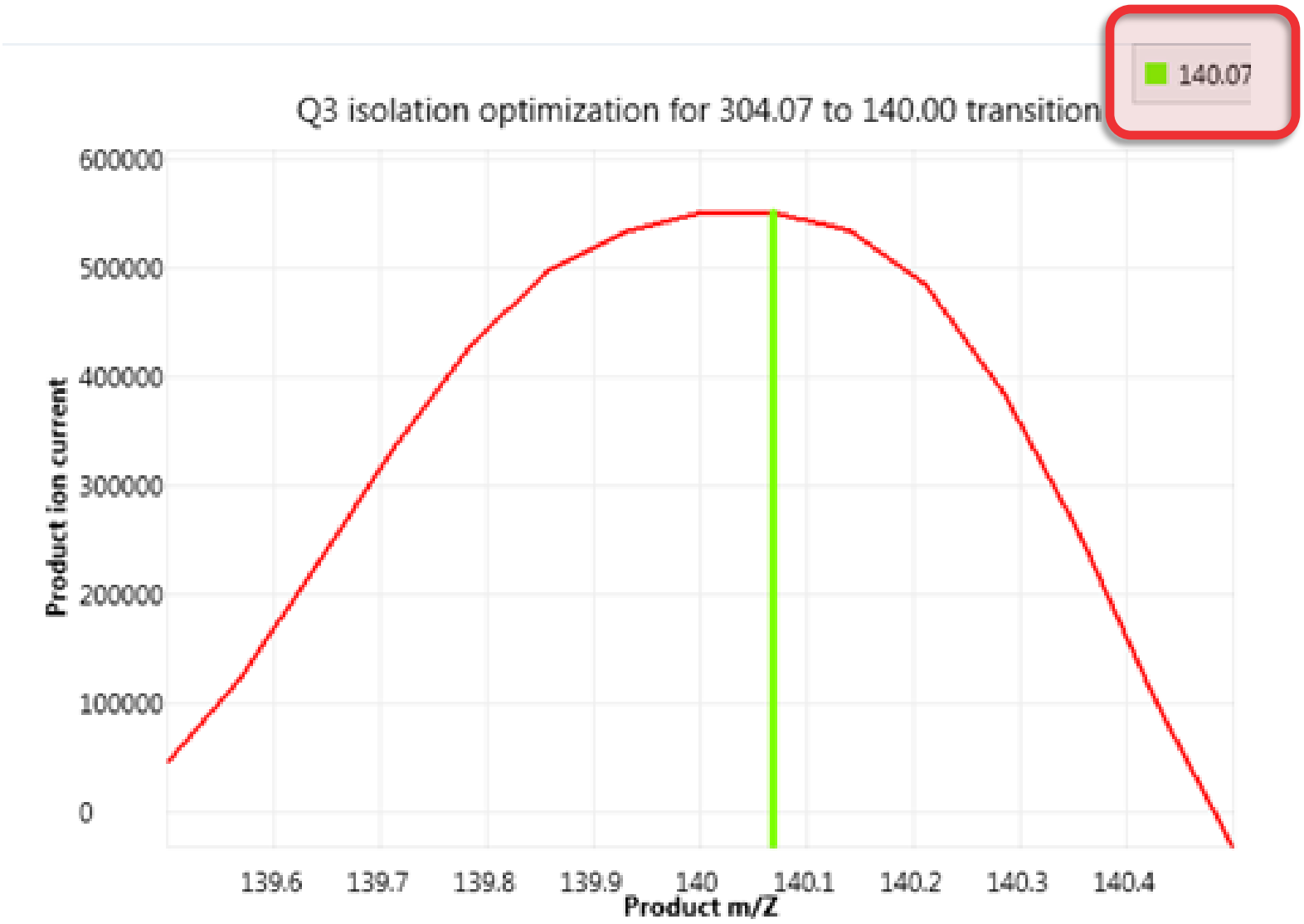
Creates Breakdown Curve



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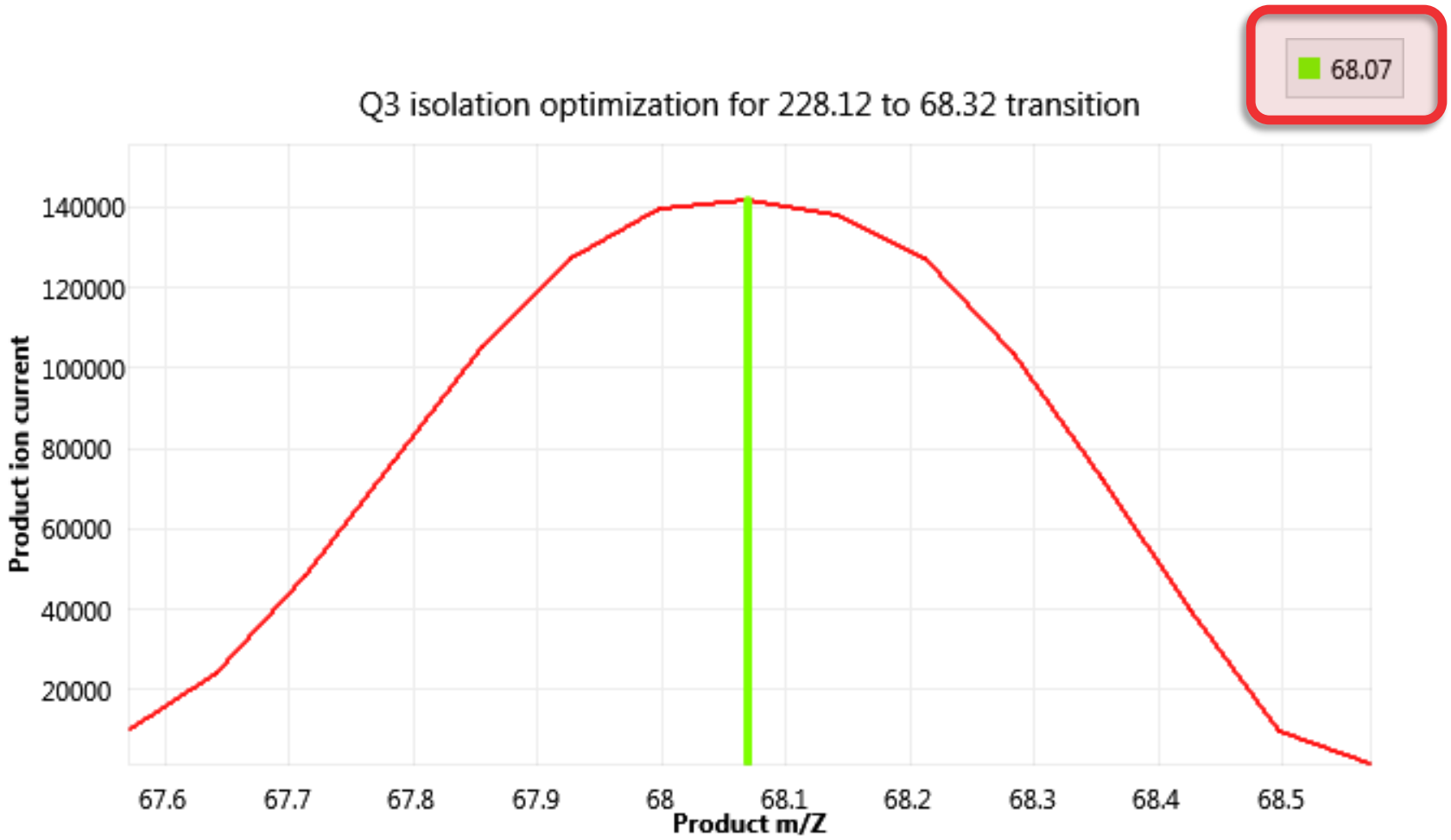
Optimizes Q3 m/z



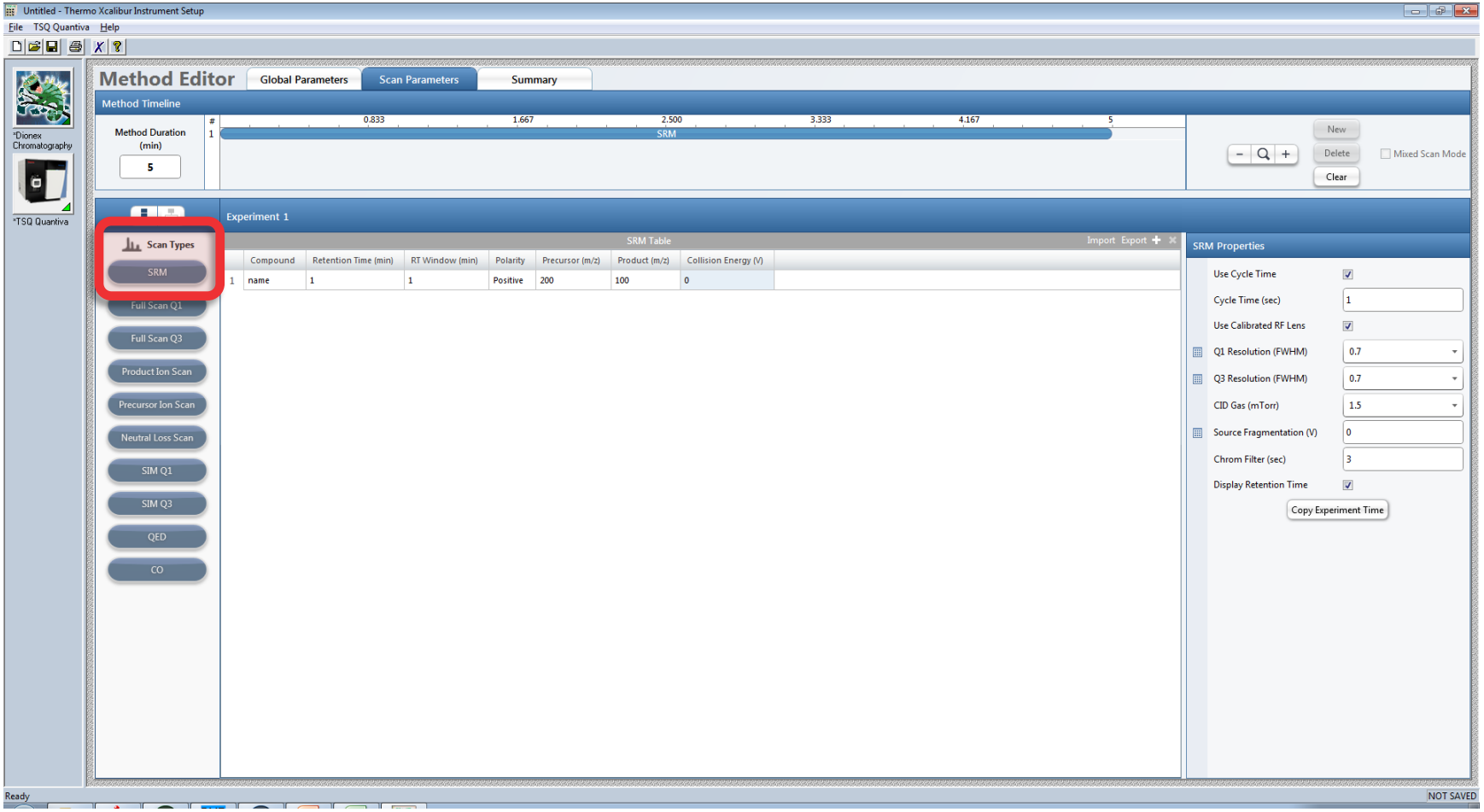
Compound Optimization Results

Optimization Results

Import Export

	Compound	Precursor (m/z)	RF Lens (V)	Product (m/z)	Collision Energy (V)	Intensity	Source Fragmentation
1	Norflurazon	304.07	110.584	160.071	34.219	649623.394	0
2	Norflurazon	304.07	110.584	264.021	29.062	182077.557	0
3	Norflurazon	304.07	110.584	87.99	46.961	353444.207	0
4	Norflurazon	304.07	110.584	284.04	24.713	4002125.111	0
5	Norflurazon	304.07	110.584	140.03	39.225	529835.62	0

Drag and Drop SRM Scan



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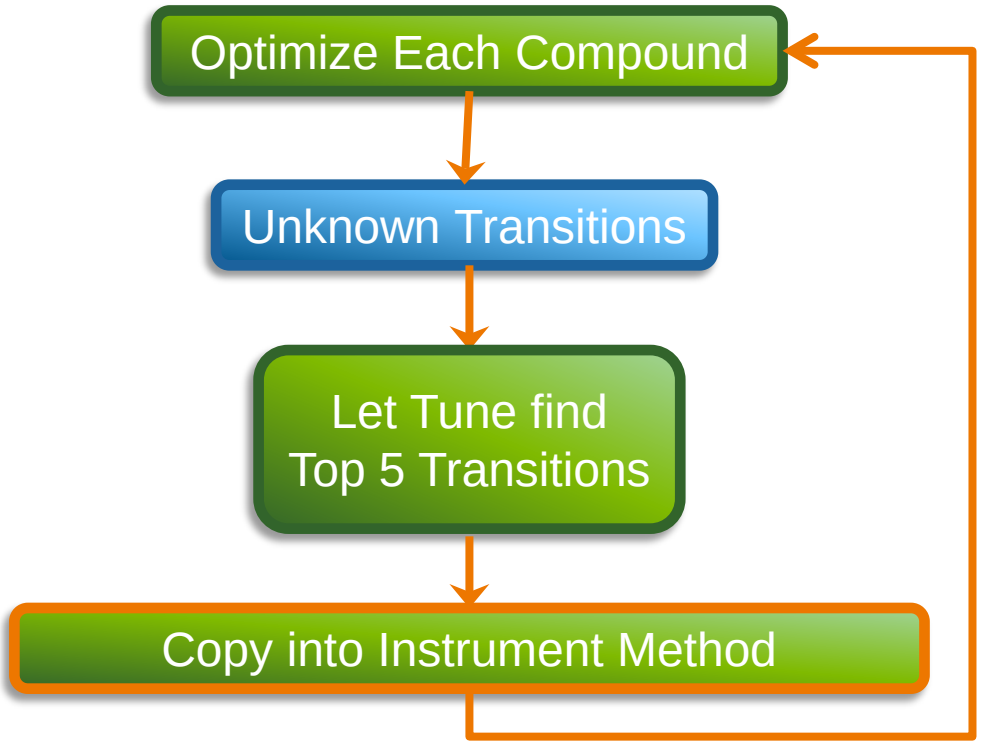
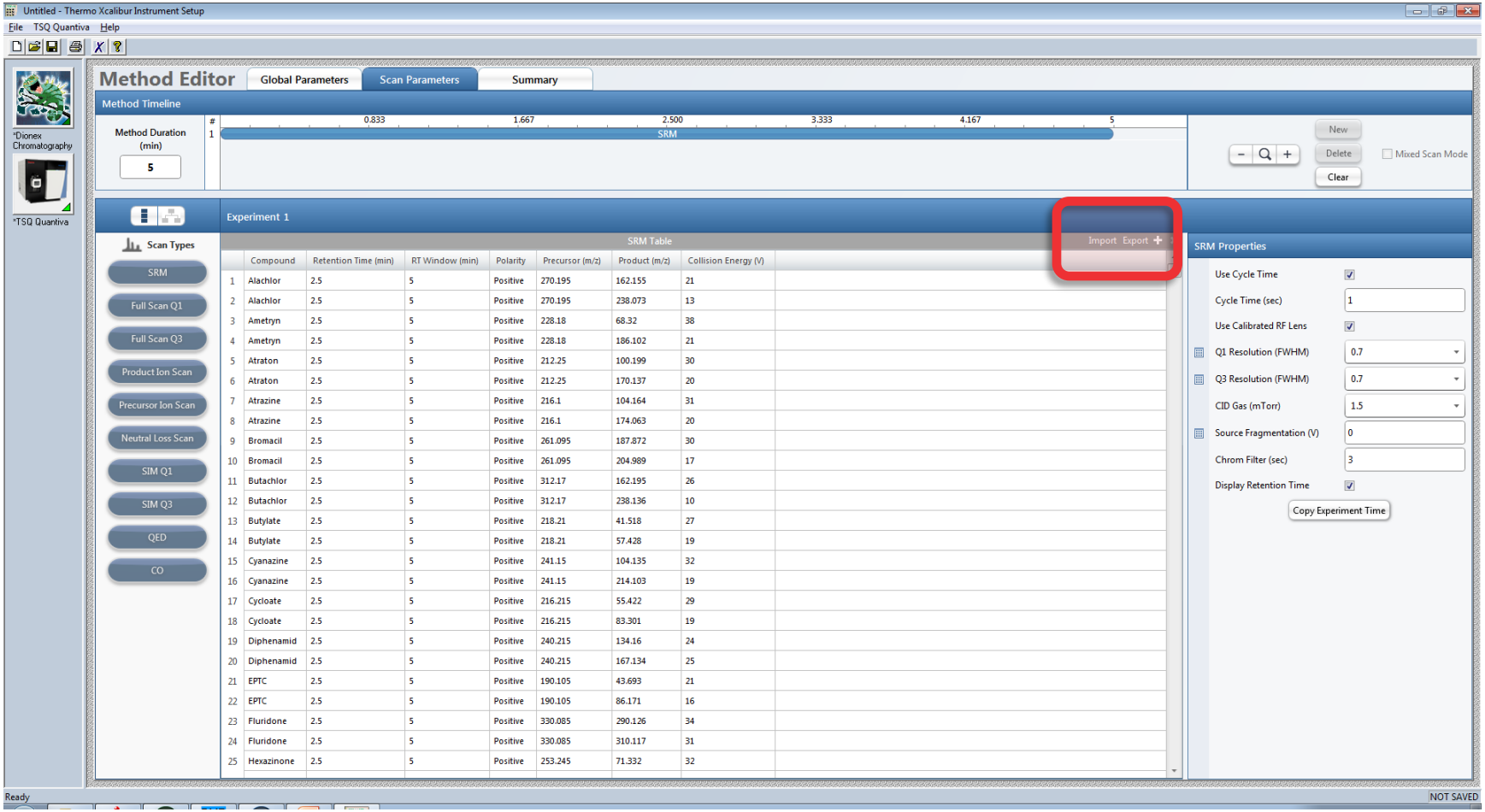
Repeat for Each Compound



Import the SRM Table



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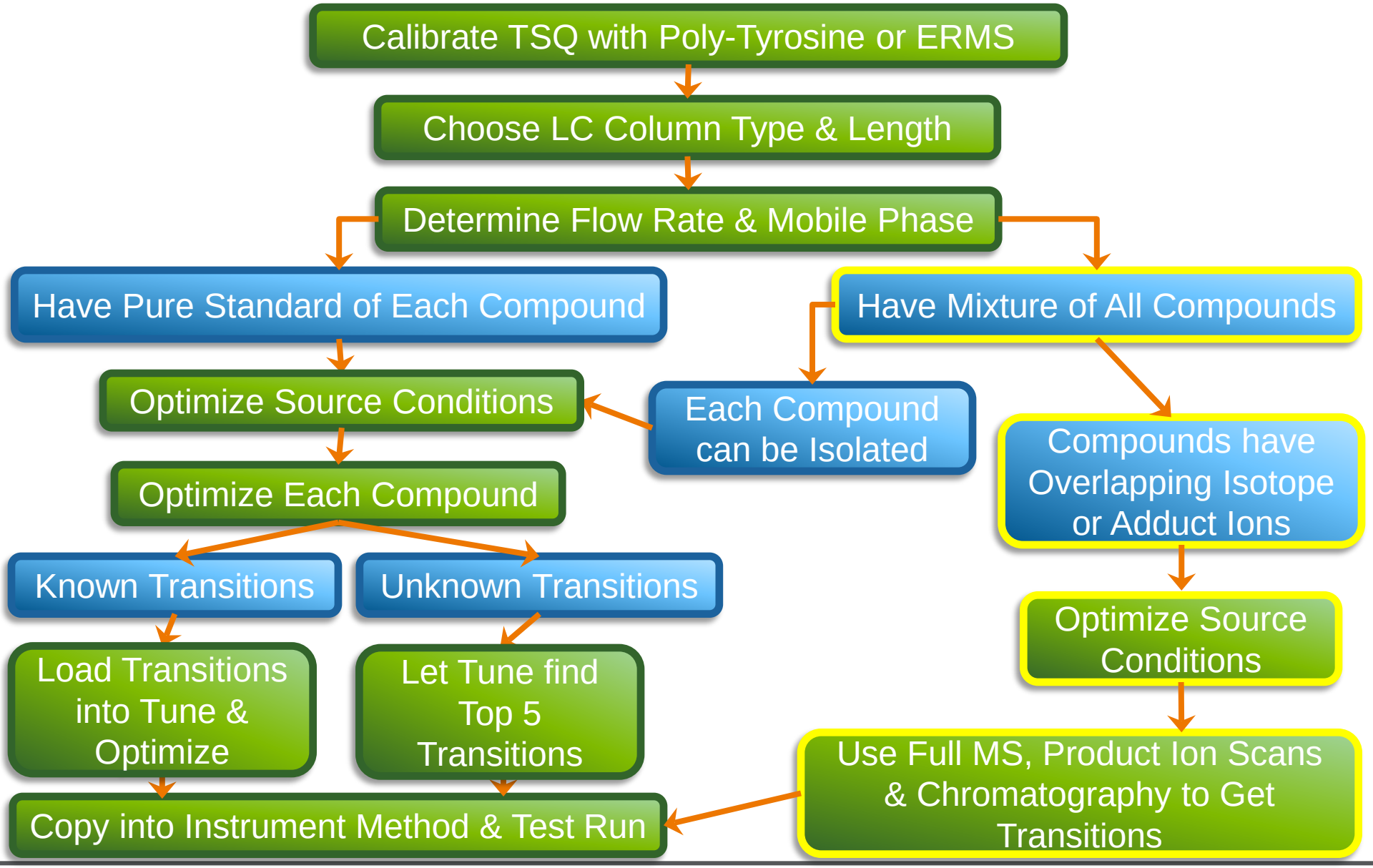
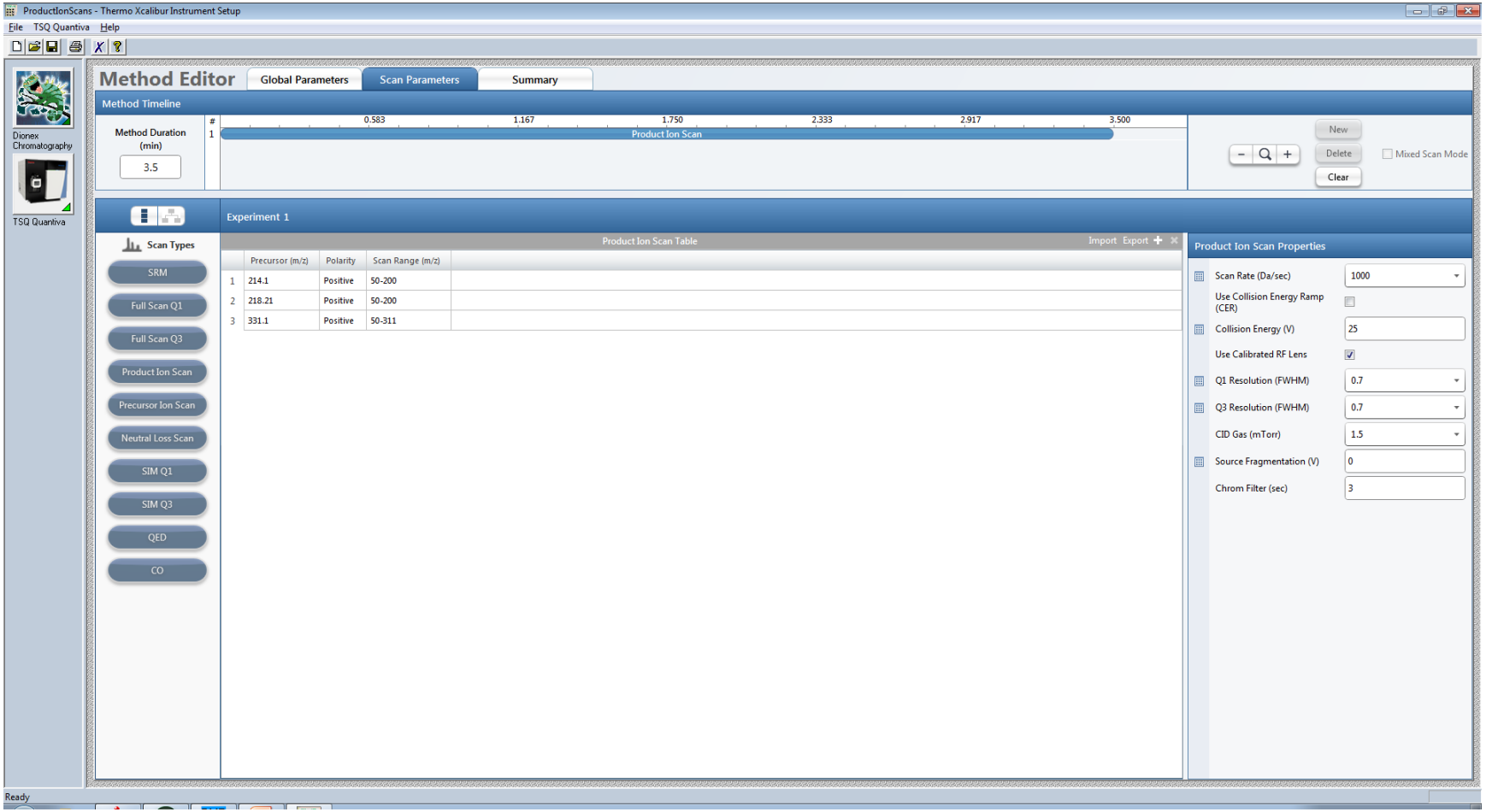
Using an LCMS Method to Find Missing Compounds



Using Product Ions Scans to Find Missing SRMs



Use Full MS, Product Ion Scans & Chromatography to Get Transitions

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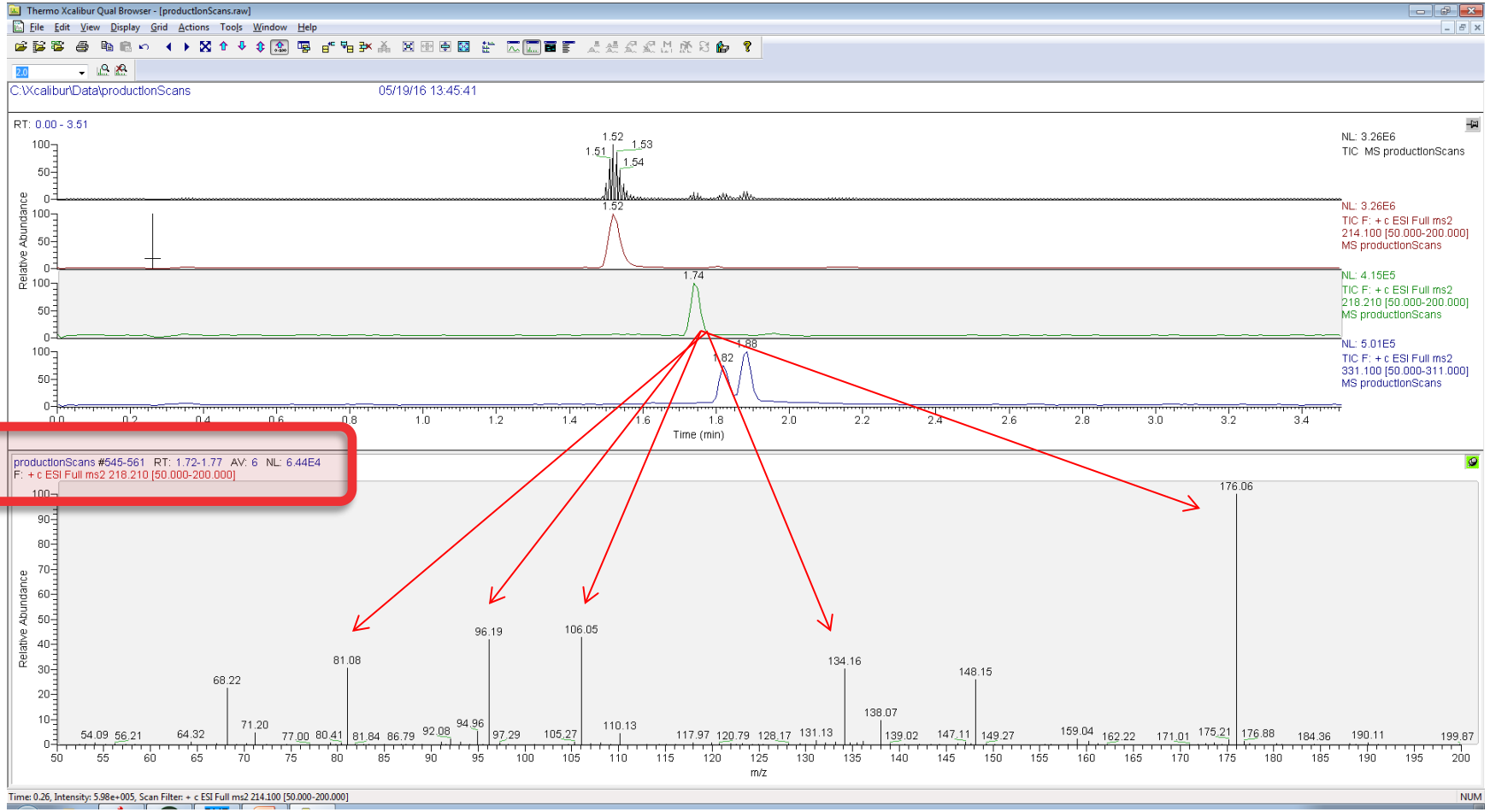
Finding Ions to Add



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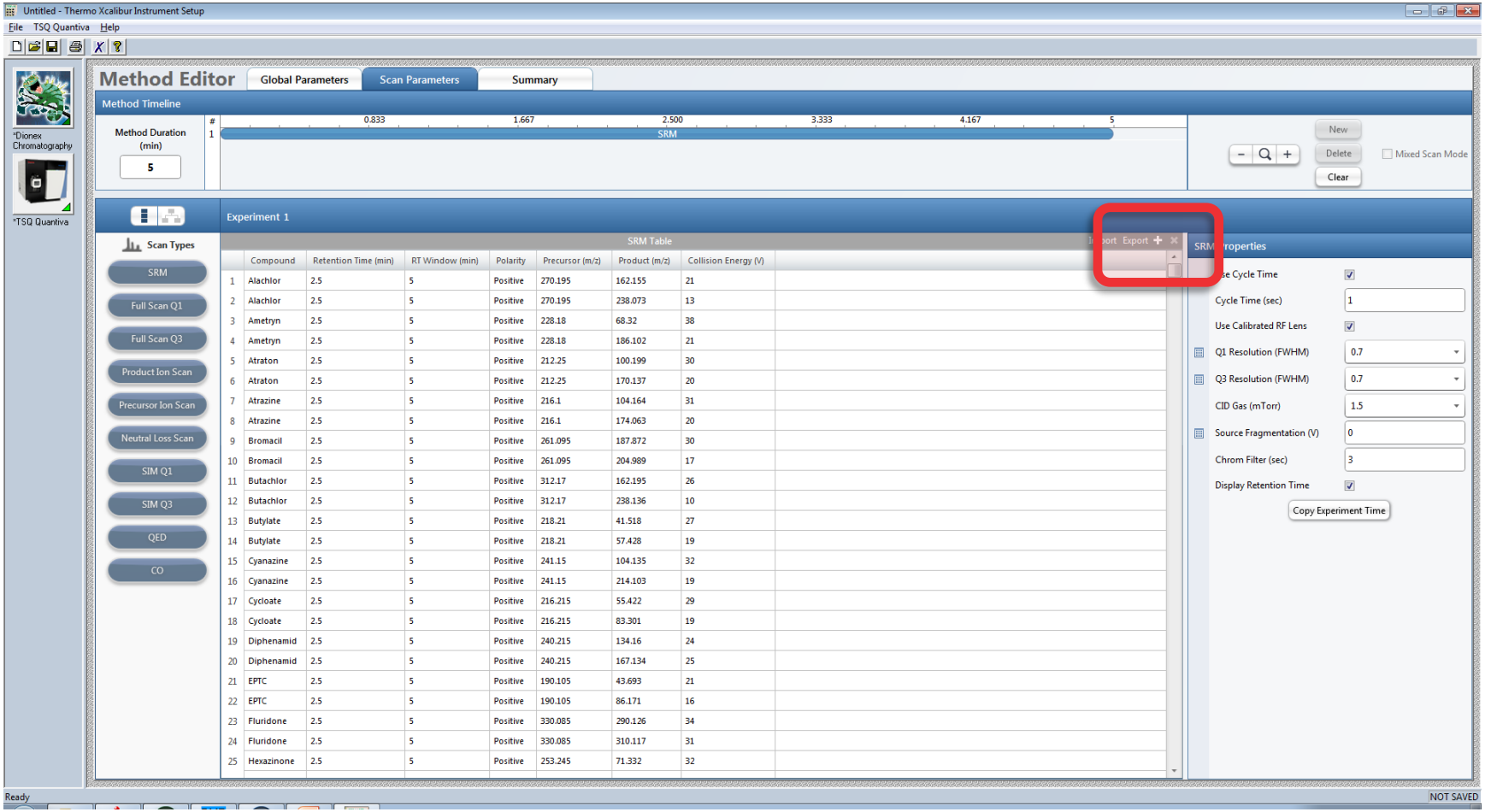
Adding the New Compounds into the Method



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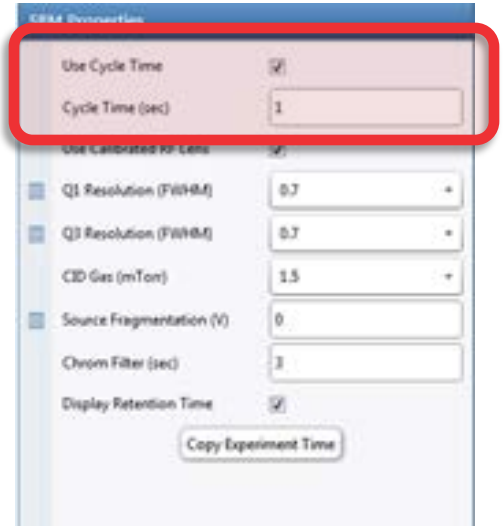
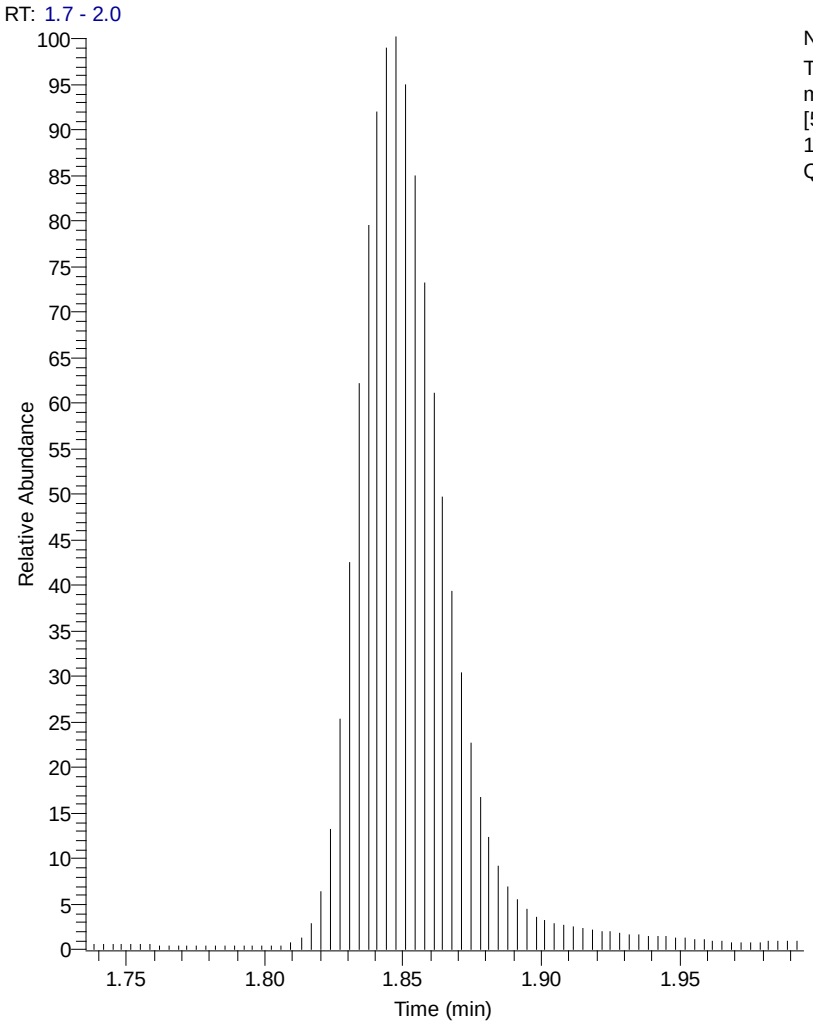
SRM Properties – Cycle Time





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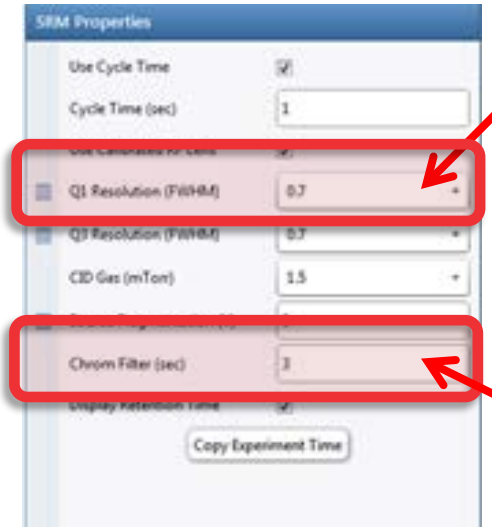
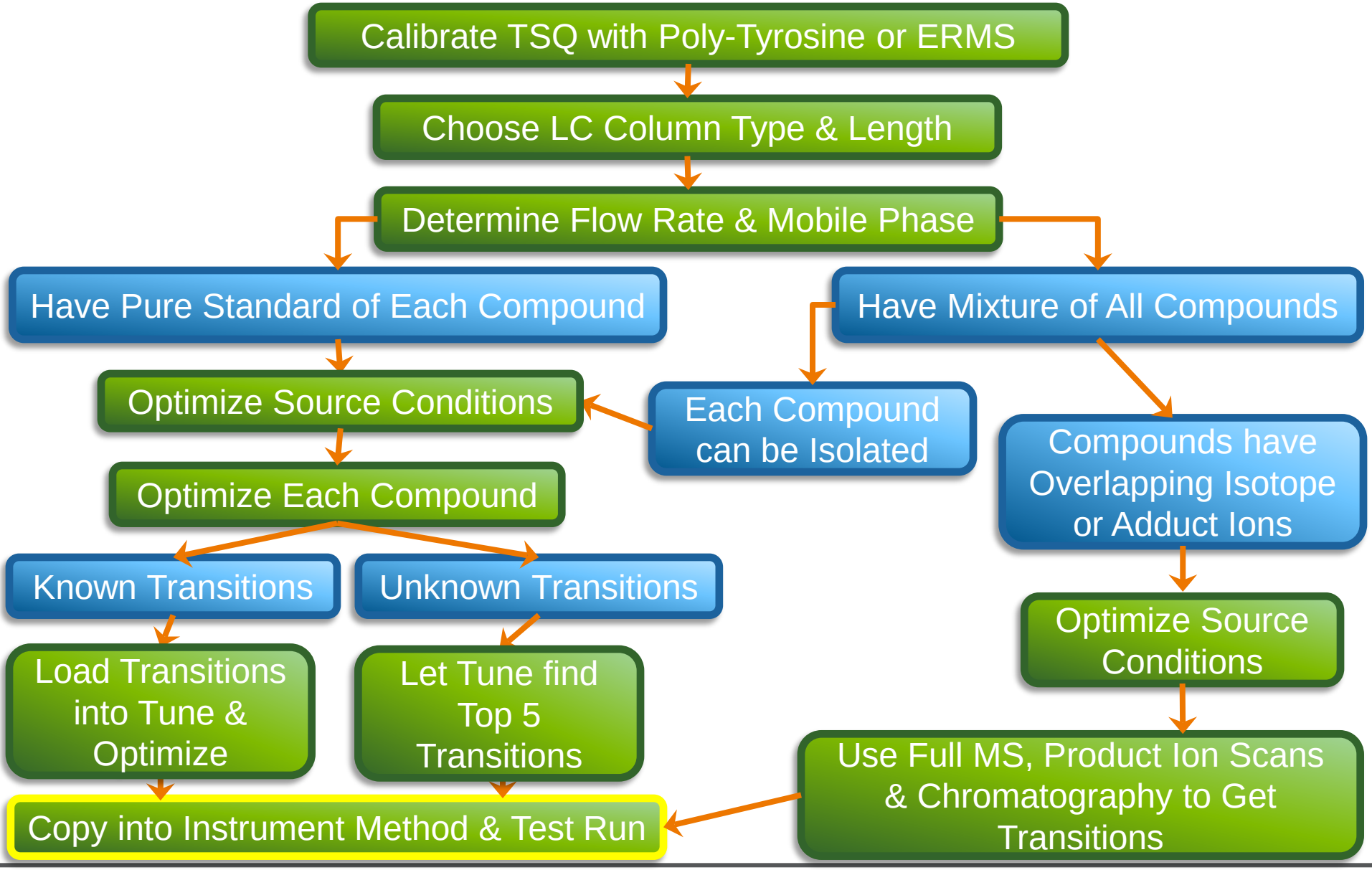
Q1 Resolution and Chrom Filter



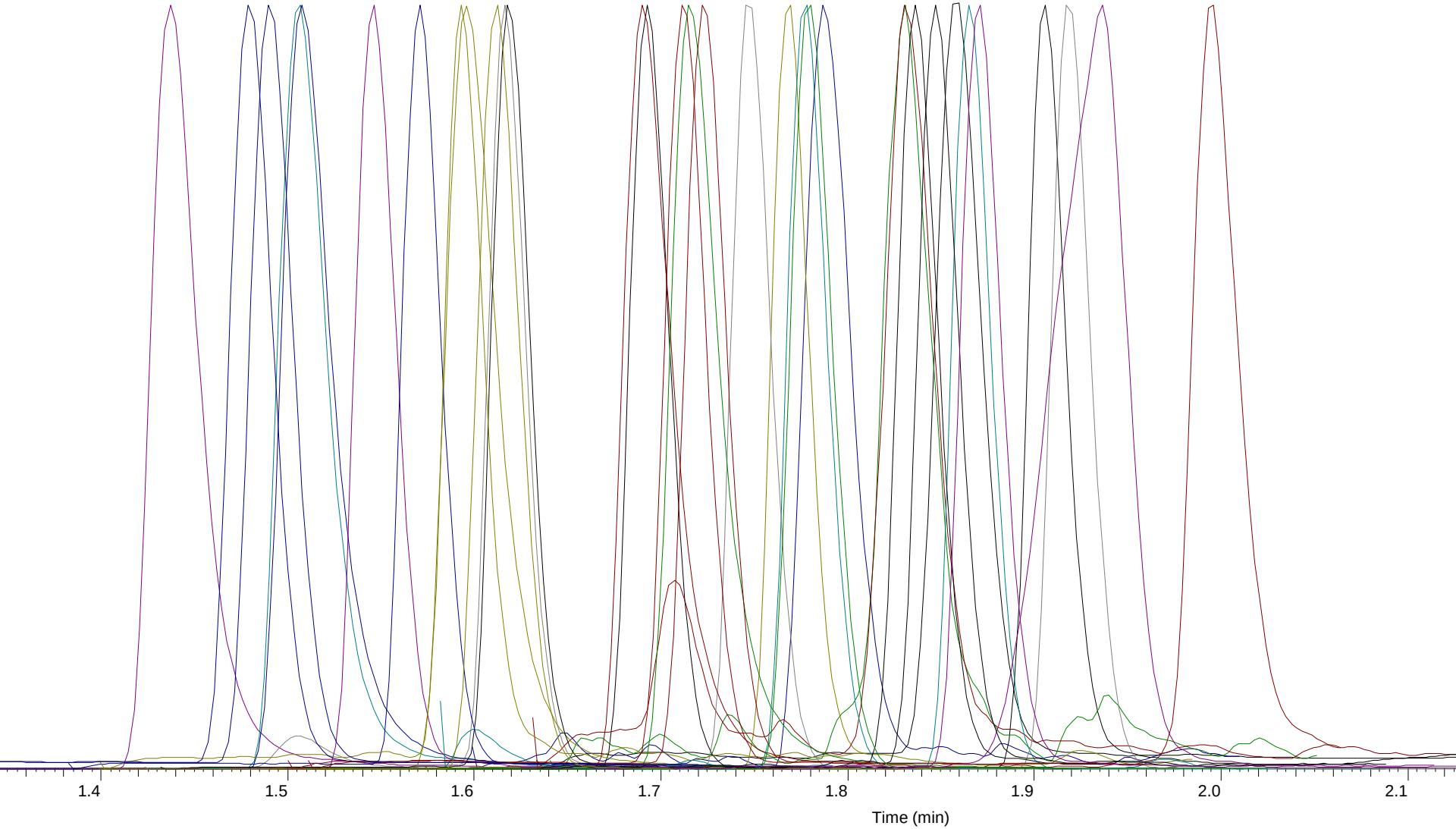
The resolution of a quadrupole is the width of mass ranges which pass through the quadrupole filter. Generally, we start off with a large width like 0.7 (gives higher signal heights) and then narrow the width when looking at a low level standard in a proper sample matrix. The narrowing only increases signal-to-noise ratios if an interference is present that the increase in mass resolution can remove.

The Chrom Filter is a set of complex algorithms which provide high and low frequency filtering common to all triple quadrupoles. You need to enter the LC full width at half height in seconds.

Test the final LC-MS/MS Method



Chromatogram of All Components Successfully Quantified



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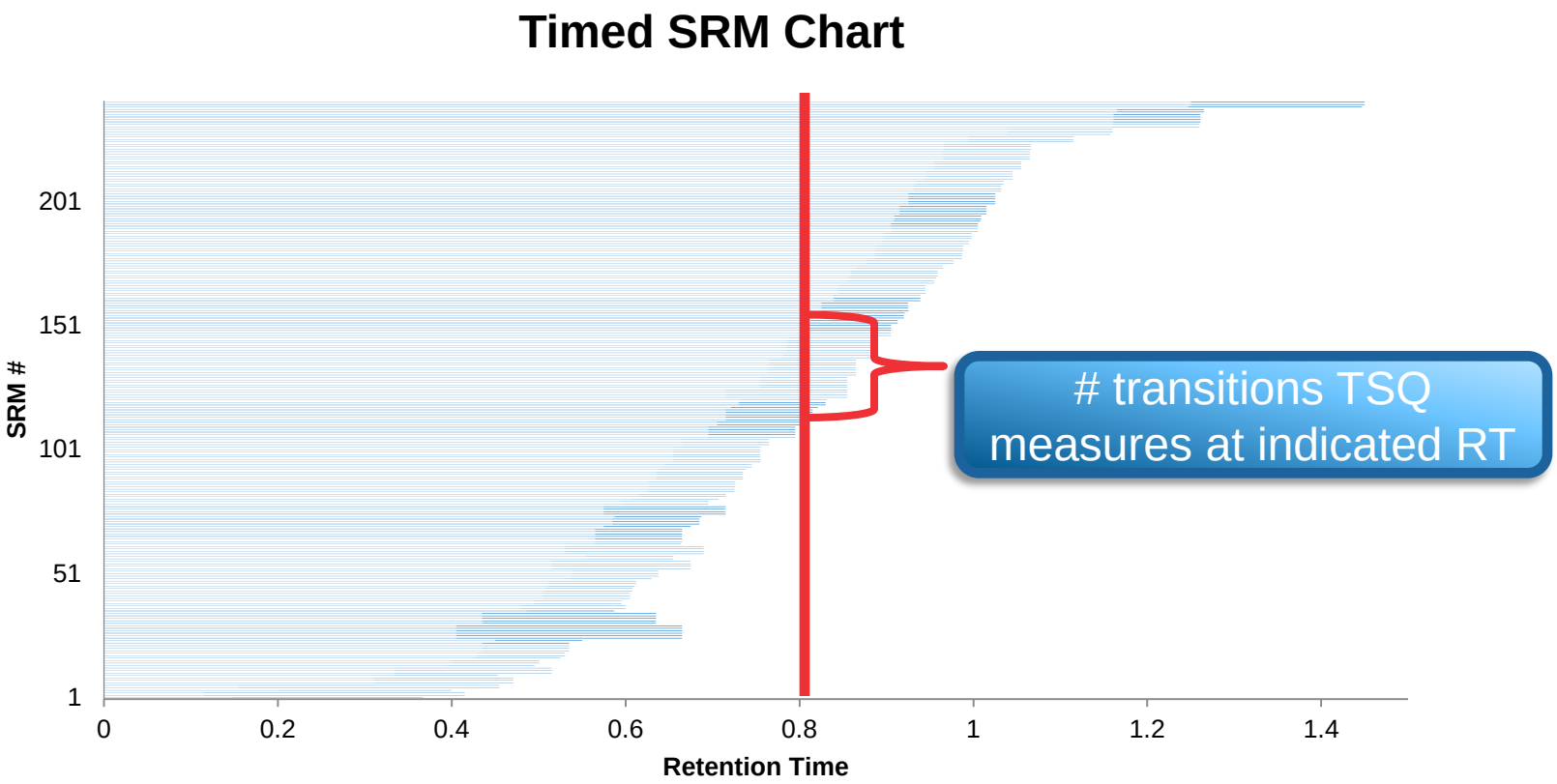
Timed SRM

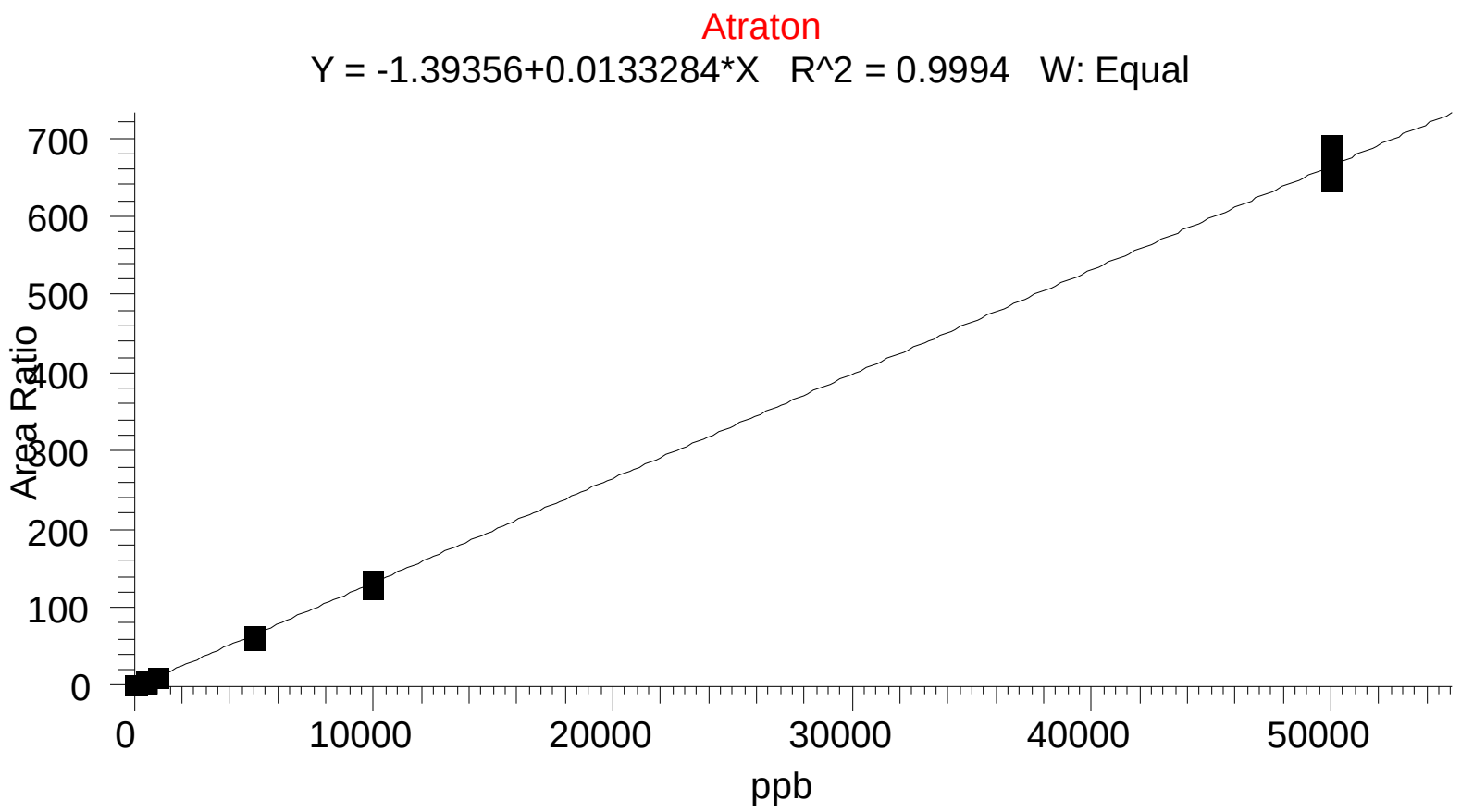


Scan Density Affects Dwell Times

Quantiva_Pesticides20 #1 RT: 1.1502
Total Ion Current: 1823.19
Scan Low Mass: 99.10
Scan High Mass: 126.10
Scan Start Time (min): 1.70
Scan Number: 2143
Base Peak Intensity: 1417.94
Base Peak Mass: 126.10
Scan Mode: + c ESI SRM ms2 223.100 [99.099-99.101, 126.099-126.101]

TSQ Quantiva Data:
==========
Elapsed Scan Time (sec): 0.006
Average Scan by Inst: Yes
Micro Scan Count: 1

Calibration Curve



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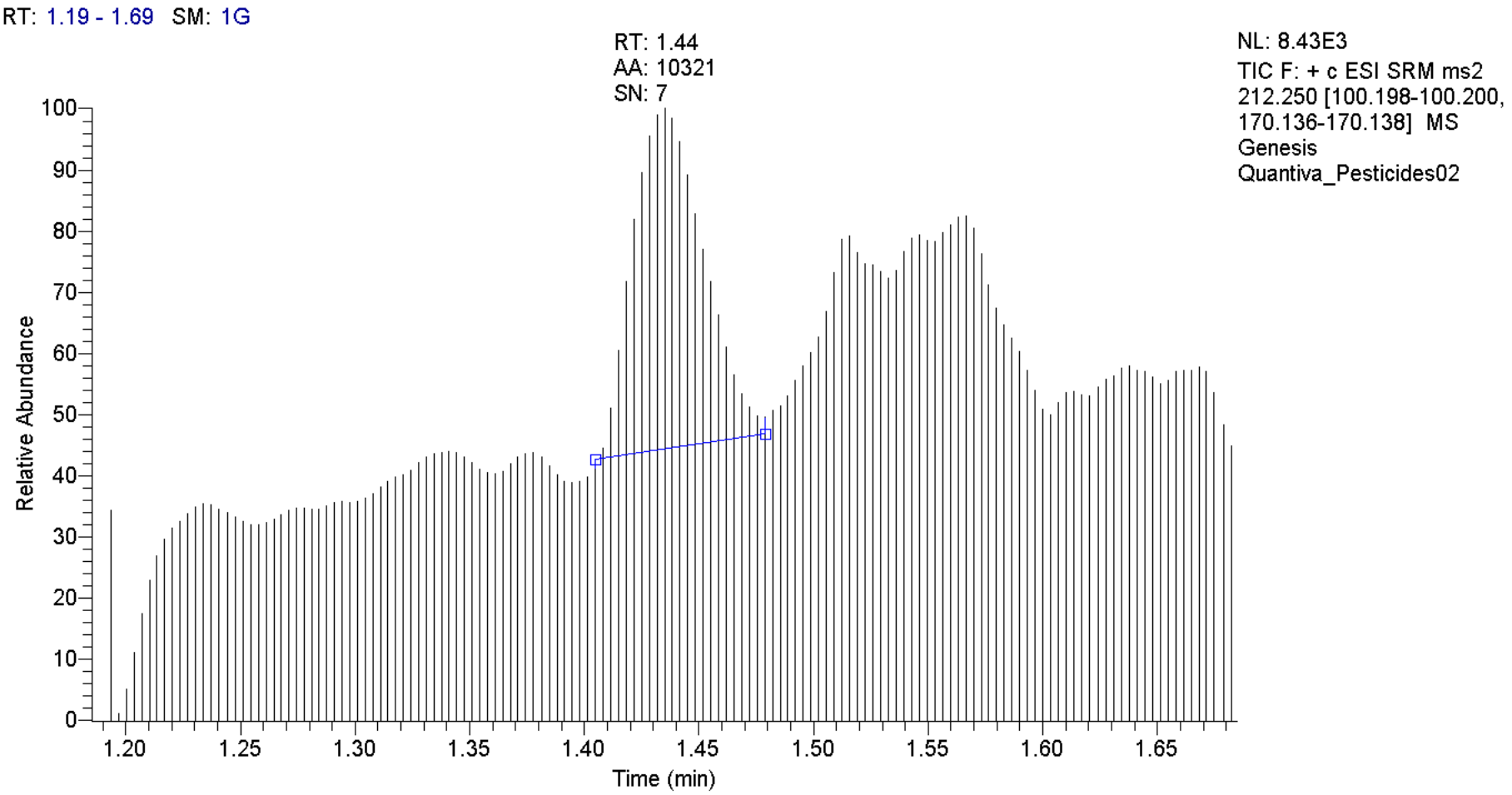
LOQ of Atraton (14.7% RSD)



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Evaluating Atraton LOQ (RSD Less than 20%, 6 injections)

Atraton				
Filename	Specified Amount	Calculated Amount	%Diff	%RSD-AMT
Quantiva_Pesticides02	1.000	0.921	-8%	14.7%
Quantiva_Pesticides03	1.000	0.956	-4%	14.7%
Quantiva_Pesticides04	1.000	0.907	-9%	14.7%
Quantiva_Pesticides05	5.000	4.676	-6%	8.5%
Quantiva_Pesticides06	5.000	5.269	5%	8.5%
Quantiva_Pesticides07	5.000	4.328	-13%	8.5%
Quantiva_Pesticides08	10.000	8.011	-20%	7.1%
Quantiva_Pesticides09	10.000	8.725	-13%	7.1%
Quantiva_Pesticides10	10.000	8.924	-11%	7.1%
Quantiva_Pesticides11	50.000	43.120	-14%	2.7%
Quantiva_Pesticides12	50.000	42.678	-15%	2.7%
Quantiva_Pesticides13	50.000	45.200	-10%	2.7%
Quantiva_Pesticides14	100.000	81.997	-18%	1.2%
Quantiva_Pesticides15	100.000	84.390	-16%	1.2%
Quantiva_Pesticides16	100.000	83.189	-17%	1.2%
Quantiva_Pesticides17	500.000	401.903	-20%	6.5%
Quantiva_Pesticides18	500.000	426.094	-15%	6.5%
Quantiva_Pesticides19	500.000	434.764	-13%	6.5%
Quantiva_Pesticides20	1000.000	1018.155	2%	1.5%
Quantiva_Pesticides21	1000.000	1023.535	2%	1.5%
Quantiva_Pesticides22	1000.000	1045.857	5%	1.5%
Quantiva_Pesticides23	5000.000	5595.561	12%	1.9%
Quantiva_Pesticides24	5000.000	5625.761	13%	1.9%
Quantiva_Pesticides25	5000.000	5725.954	15%	1.9%

Second set of injections after unknowns not shown for clarity

Conclusion

- Conclusion
  - A workflow for developing a triple quadrupole method was shown
  - Please join us for other sessions showing:
    - Maintenance of your instrument
    - Software Analysis using TraceFinder