

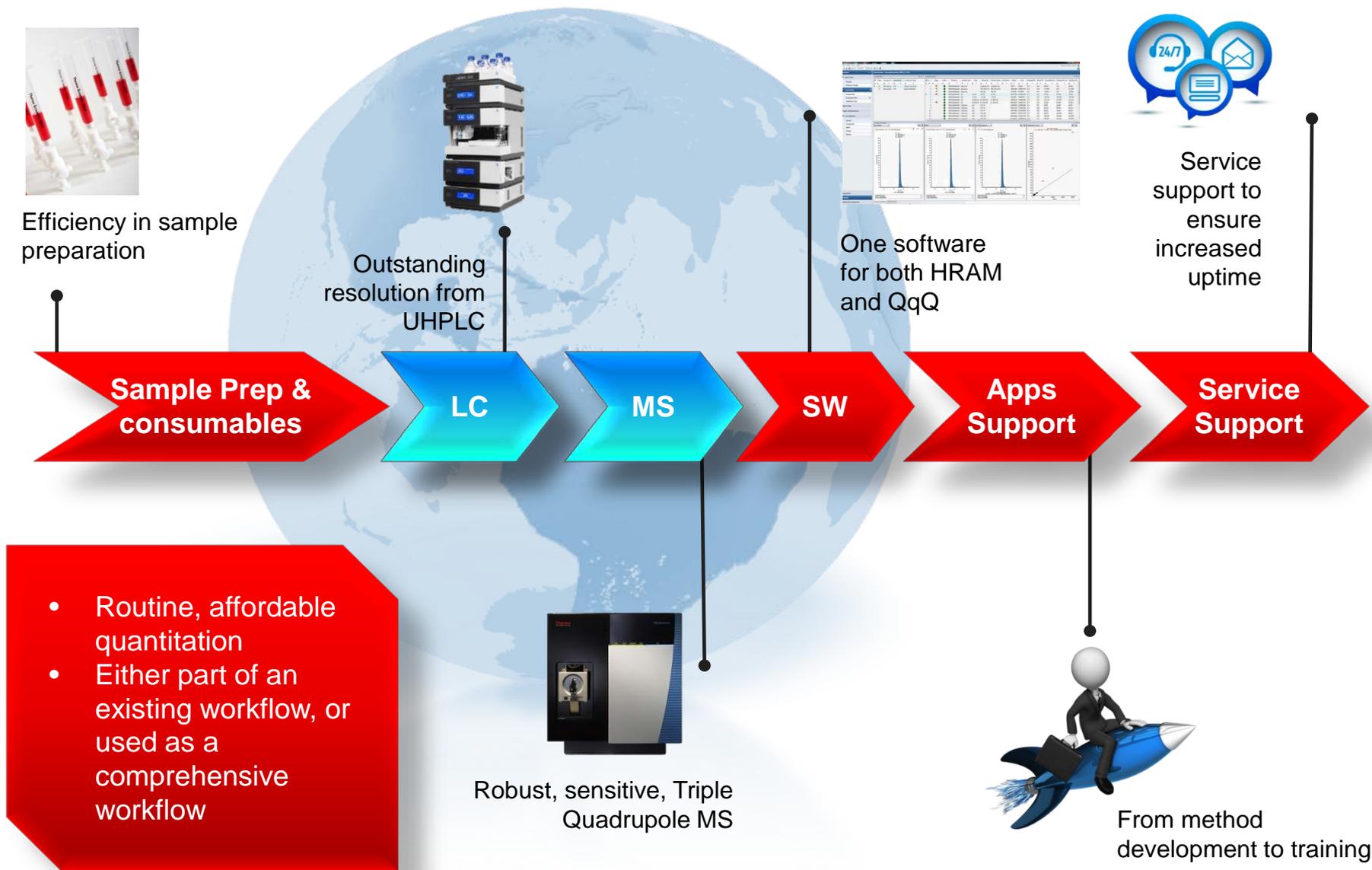


**ThermoFisher**  
S C I E N T I F I C

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

Craig Dufresne and Kevin McHale, Content Creators

# The Whole Workflow ...



# Expectation from LC-MS Technology

Hundreds of pesticides identified and quantified in one run

A comprehensive platform solution with hardware and software



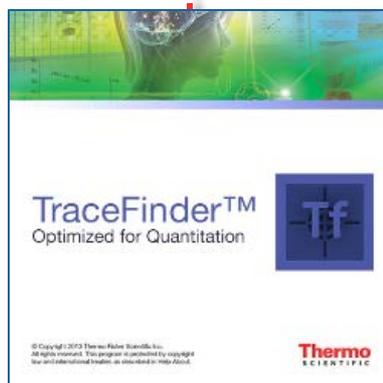
Demand for lower LLOQ with higher selectivity, specificity, and confidence

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

# Expectation from LC-MS Technology

Hundreds of pesticides identified and quantified in one run

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

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

# Pesticide Explorer – One Problem...Multiple Solutions!

| Thermo Scientific™ TSQ<br>Endura™ Triple Quadrupole MS | Thermo Scientific™ TSQ<br>Quantiva™ Triple Quadrupole<br>MS | Thermo Scientific™<br>Q Exactive™ Focus MS | Thermo Scientific™<br>Q Exactive™ Focus MS |
|--------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------|--------------------------------------------|
| Standard QUAN                                          | Premium QUAN                                                | HRAM QUAN                                  | HRAM SCREEN QUAN                           |



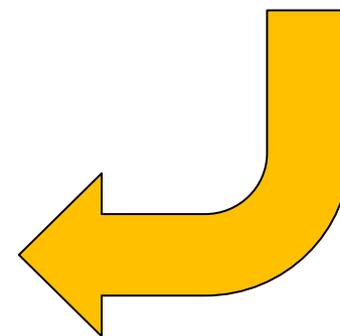
**OPTON-30425**

**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

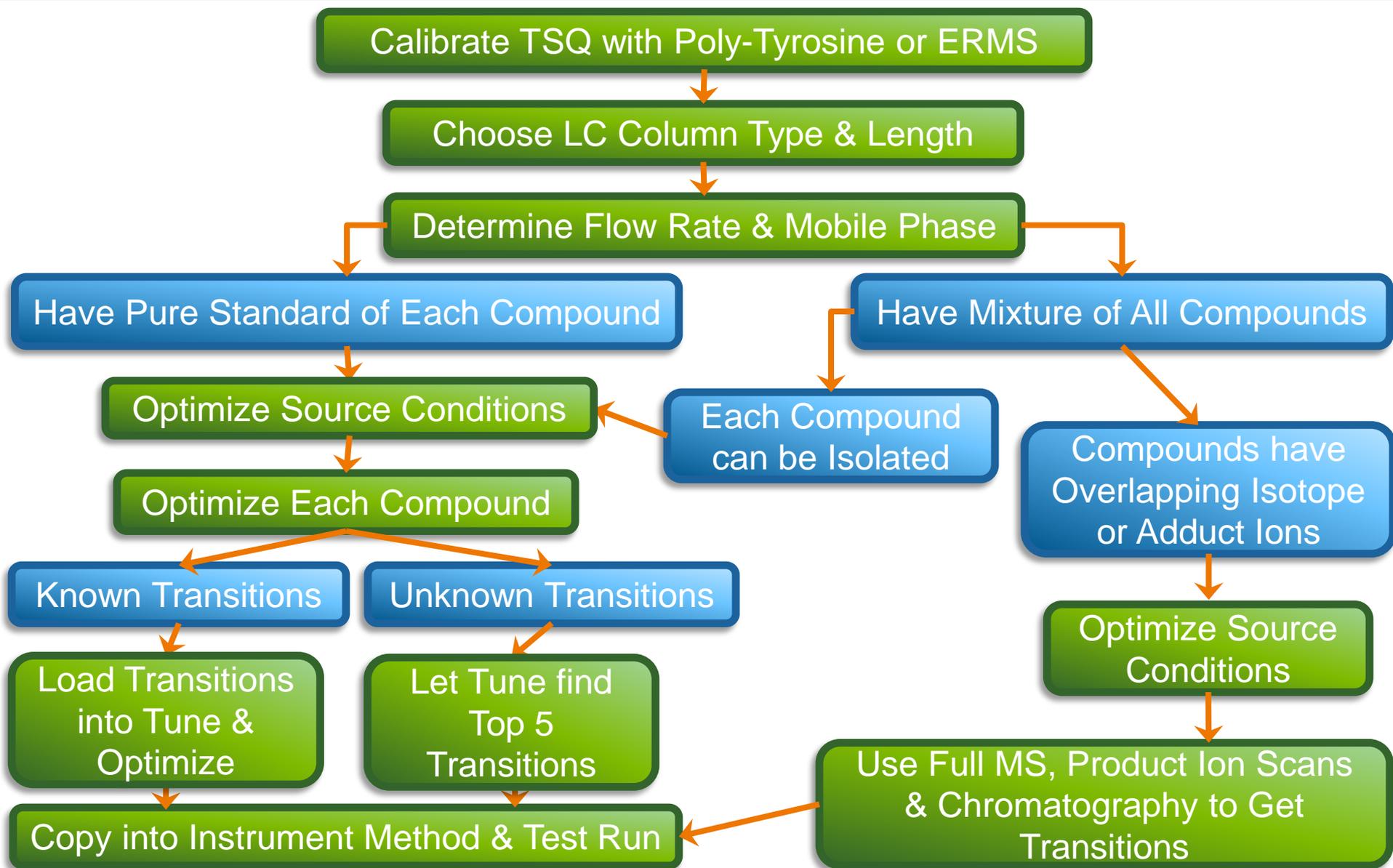
- Develop an instrument method for the separation of the Restek 525.2 Organonitrogen pesticide reference sample (Restek part number 33012) as a teaching example
- Understand the processes of *tuning*, *calibration*, *optimization*, and *method building*
- Explain which parameters in the method can be 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

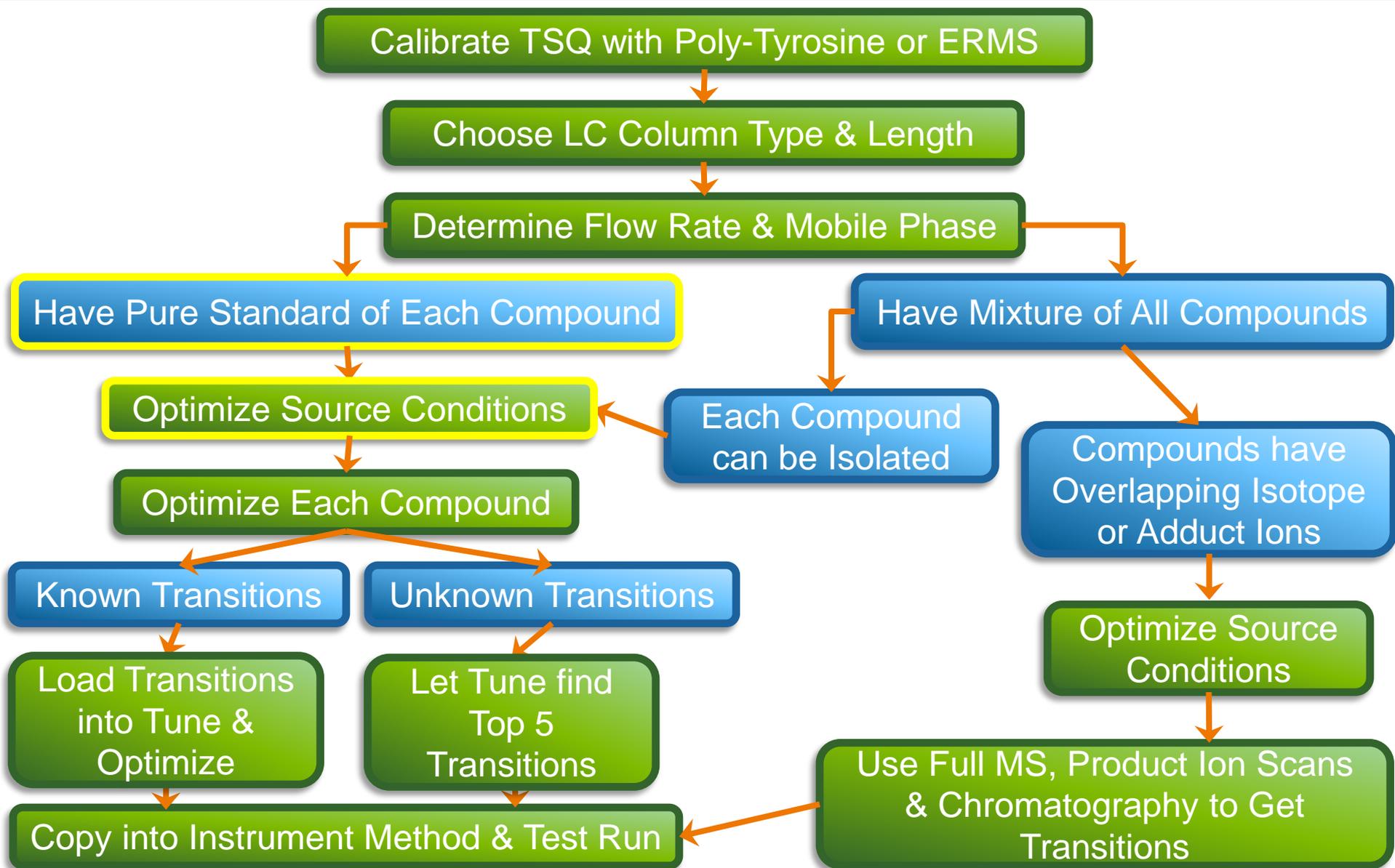
- This method was chosen to produce that fastest possible separation of the 37 component Restek 525.2 organonitrogen pesticide mix
- Ultimate 3000 RSLC with a HPG pump and a temperature controlled column compartment
- Accucore™ Vanquish™ C<sub>18</sub><sup>+</sup> 2.1 mm x 50 mm, 1.5 um (PN 27101-052130) 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

Choose LC Column Type & Length



Determine Flow Rate & Mobile Phase

# Optimizing Source Conditions When You Have Pure Compounds

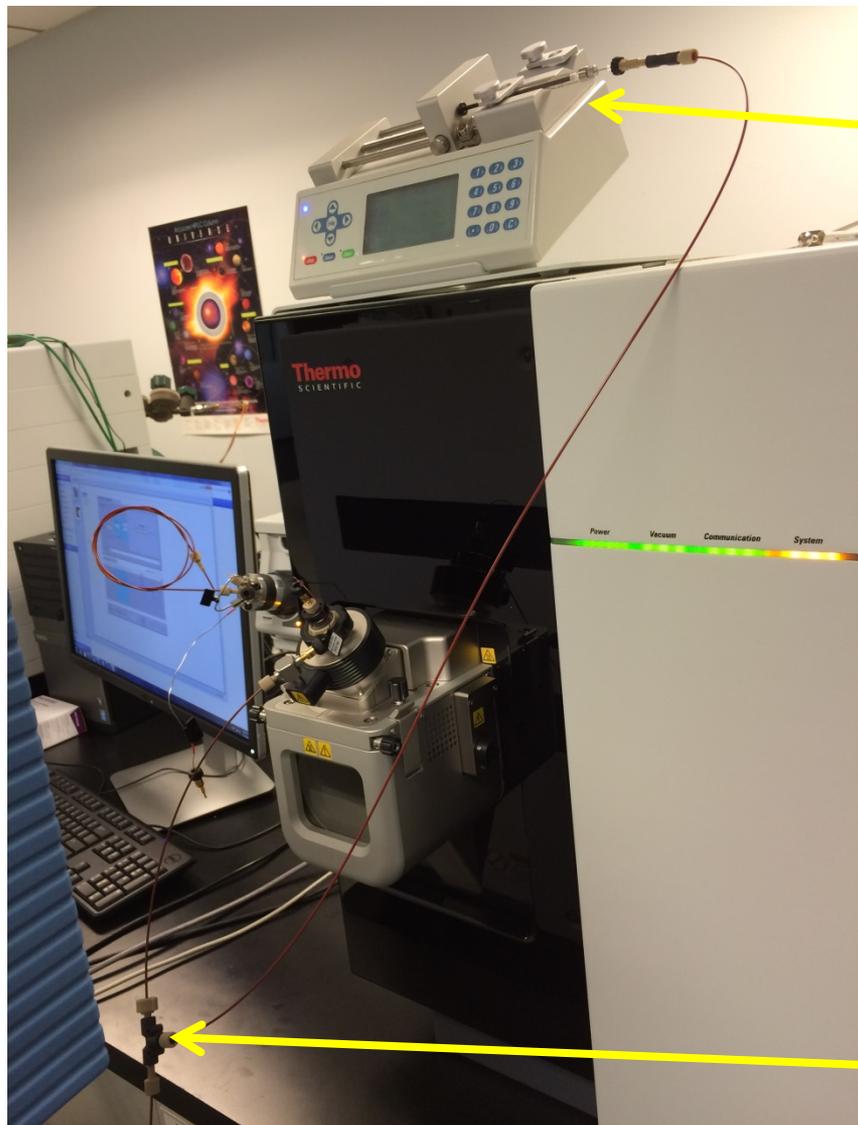


# Steps to Start Up

- 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](http://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

TSQ Quantiva Tune Application 2.0.1292.15

Valve 1-6 A  
Syringe OFF  
Record  
c:\Thermo\Data  
Rawdata \_20160518044454 View  
Changing

ION SOURCE DEFINE SCAN CALIBRATION

Ion Source Optimization

Current LC Flow (µL/min) 400 Get Defaults

Ion Source Type H-ESI

Pos Ion Spray Voltage (V) 4200 4136

Neg Ion Spray Voltage (V) 2500

Sheath Gas (Arb) 45 45

Aux Gas (Arb) 13 13

Sweep Gas (Arb) 1 1

Ion Transfer Tube Temp (°C) 340 337.8

Vaporizer Temp (°C) 360 337

Apply

90 80 70 60 50 40 30 20 10  
10 20 30 40 50 60 70 80 90

90 80 70 60 50 40 30 20 10  
10 20 30 40 50 60 70 80 90

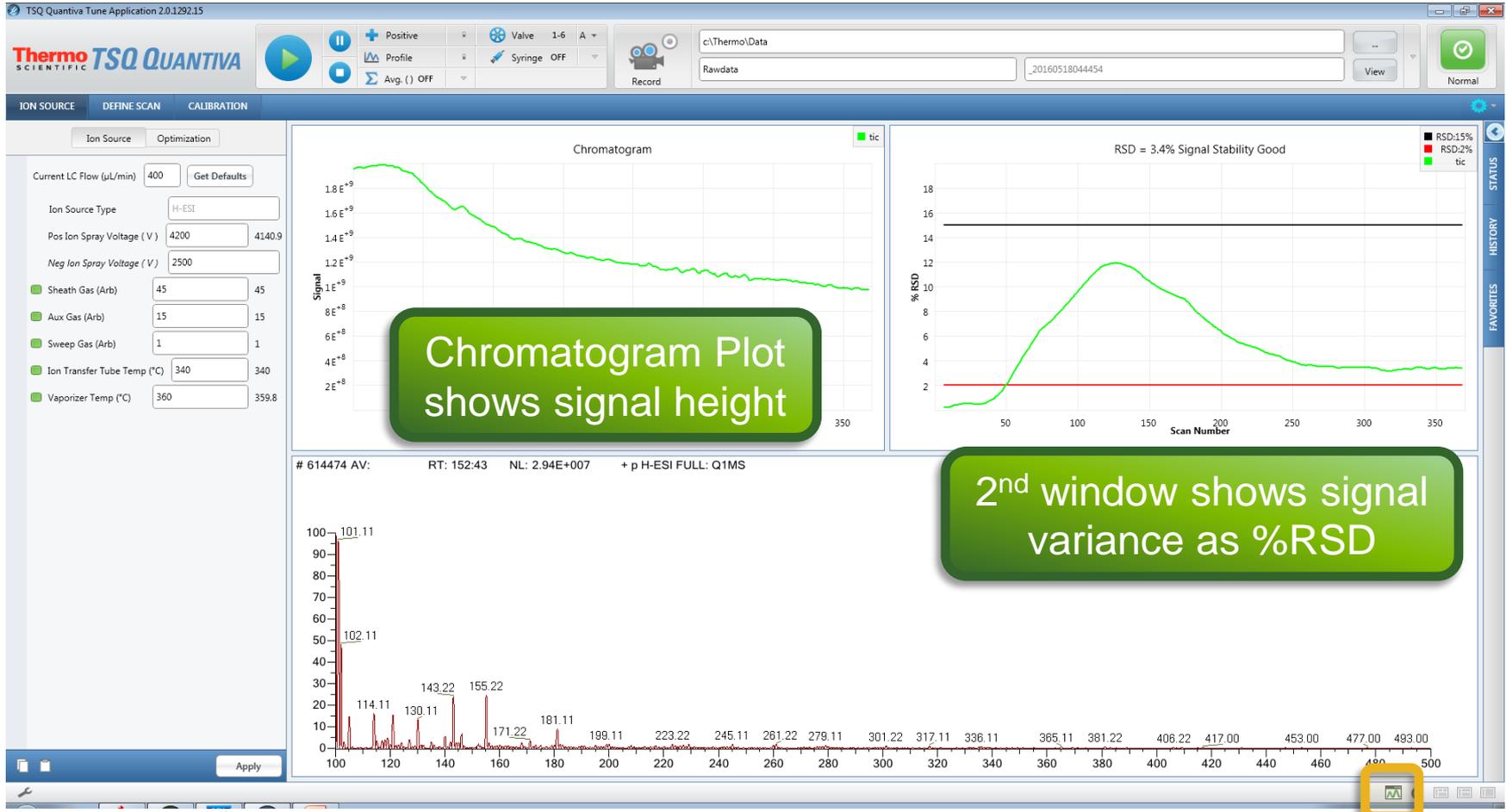
# 611931 AV: RT: 133.44 NL: 3.21E+006 + p H-ESI FULL: Q1MS

100 80 60 40 20 0  
100 150 200 250 300 350 400 450 500

145.11 163.22 201.22 229.22 257.44 285.33 299.33 371.44 391.44 429.22 479.44

FAVORITES HISTORY STATUS

# Tune Window with LC Flow Teed into the Syringe Pump

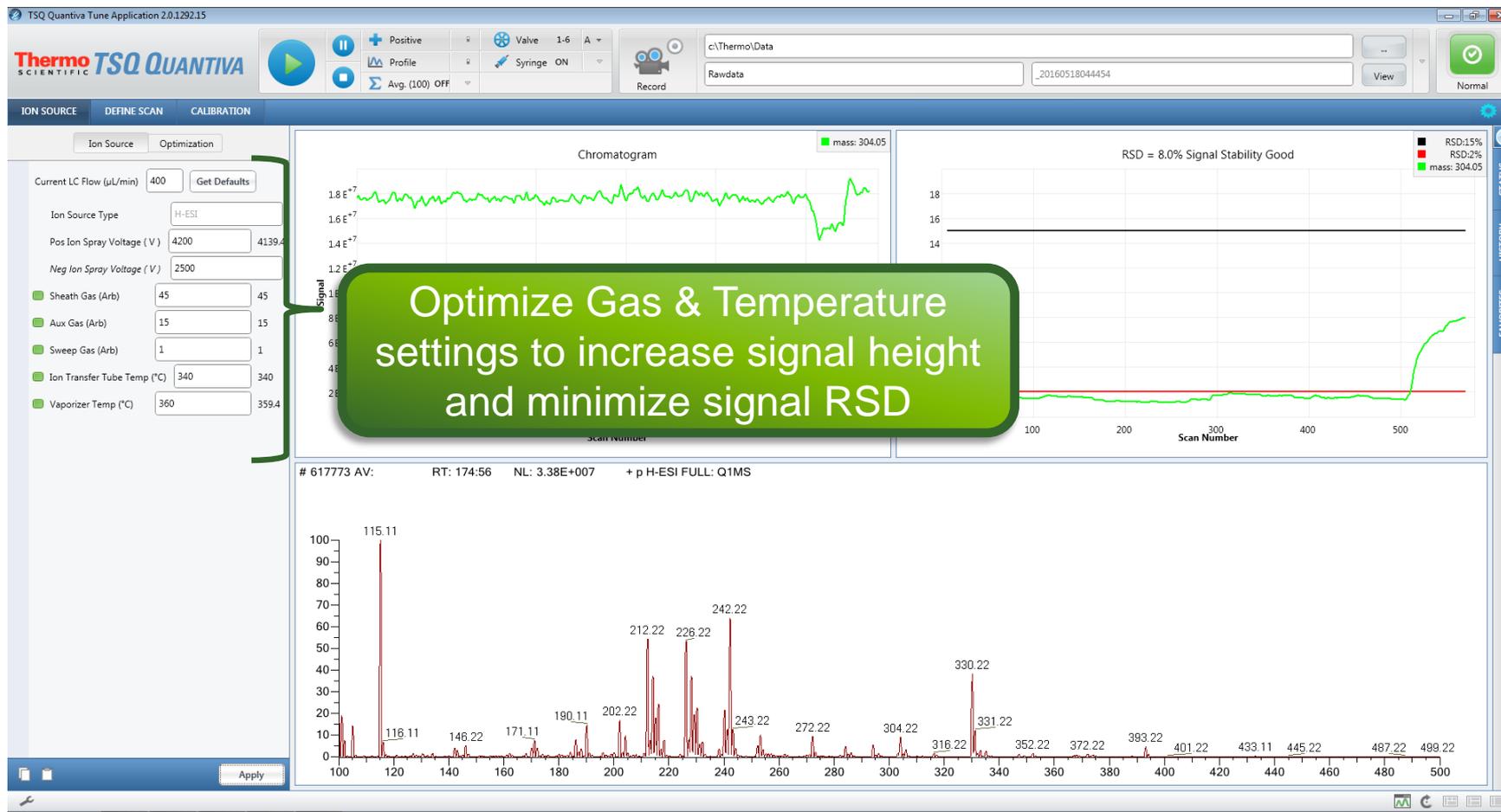


Chromatogram button

# Parameters to Optimize

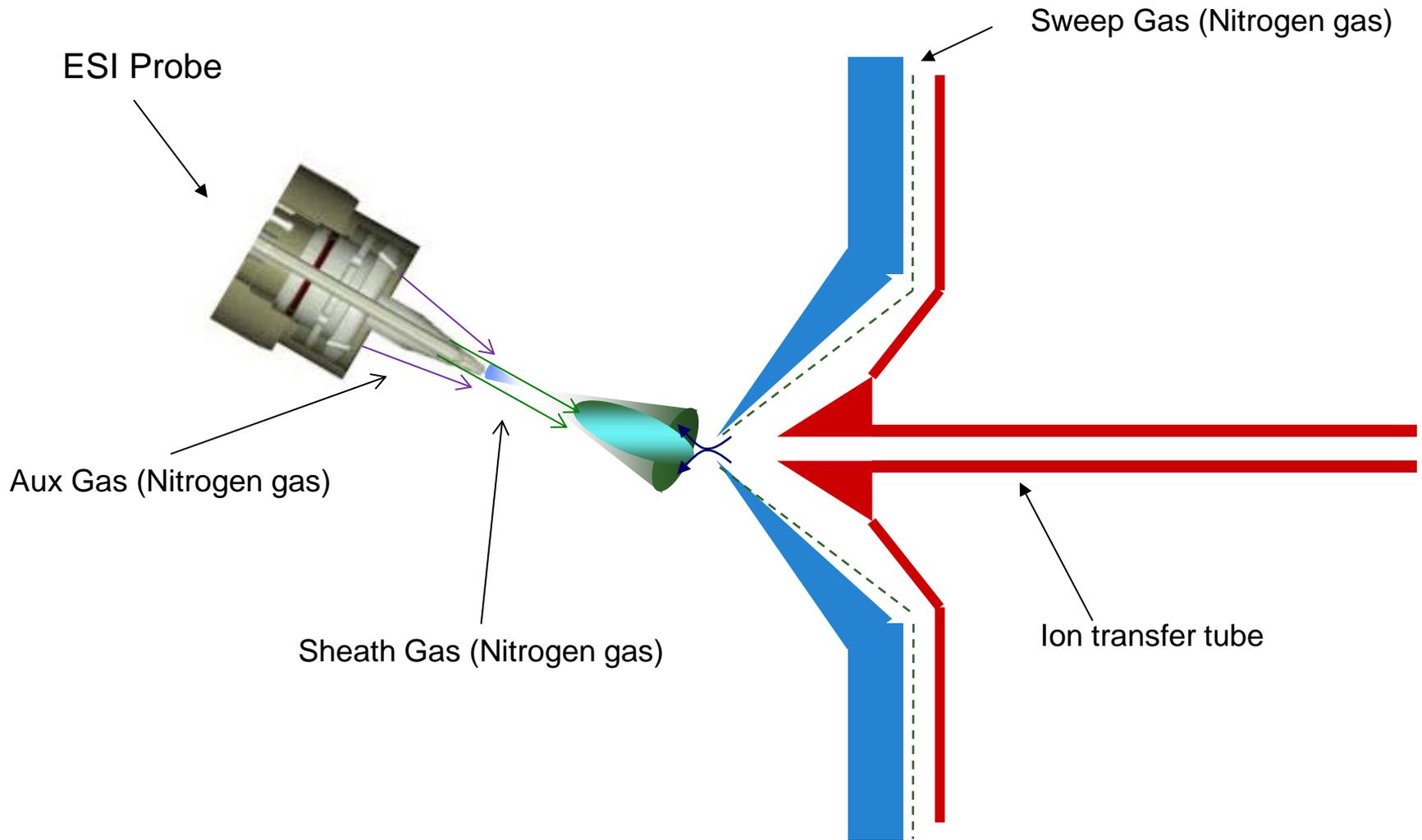
- One compound is used to optimize *ion source conditions* to make the most stable spray:
  - Probe Position
  - Sheath gas
  - Aux gas
  - Sweep gas
  - Spray Voltage
  - Source Temperatures
- Each compound is used to optimize the *mass spectrometer parameters* for the best possible response and selectivity:
  - Precursor m/z
  - RF-Lens voltage
  - Product ions / SRM transitions
  - Collision Energies
  - Ion Polarity

# Optimization of Default Source Conditions Manually

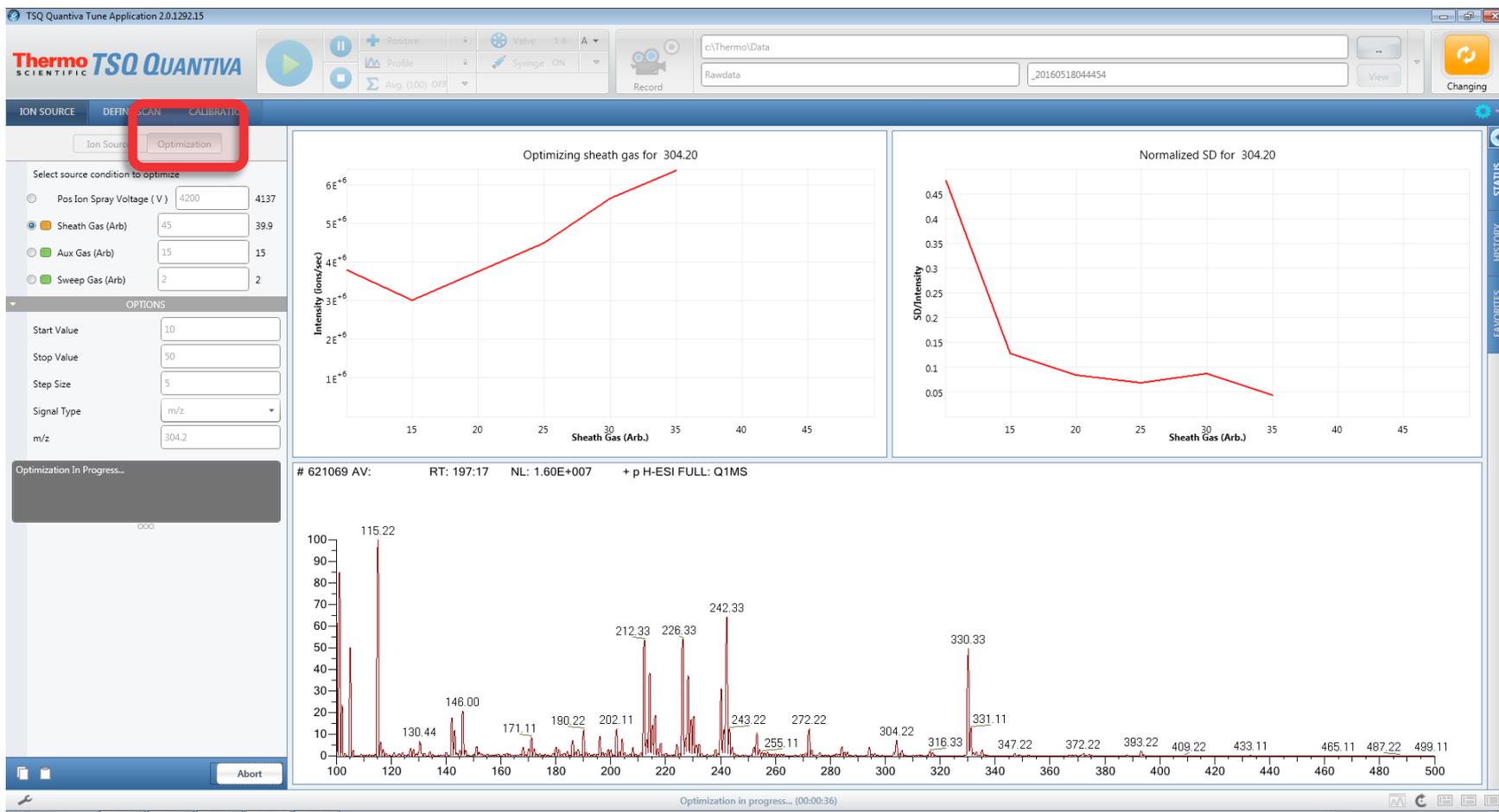


Optimize Gas & Temperature settings to increase signal height and minimize signal RSD

# Probe Gases

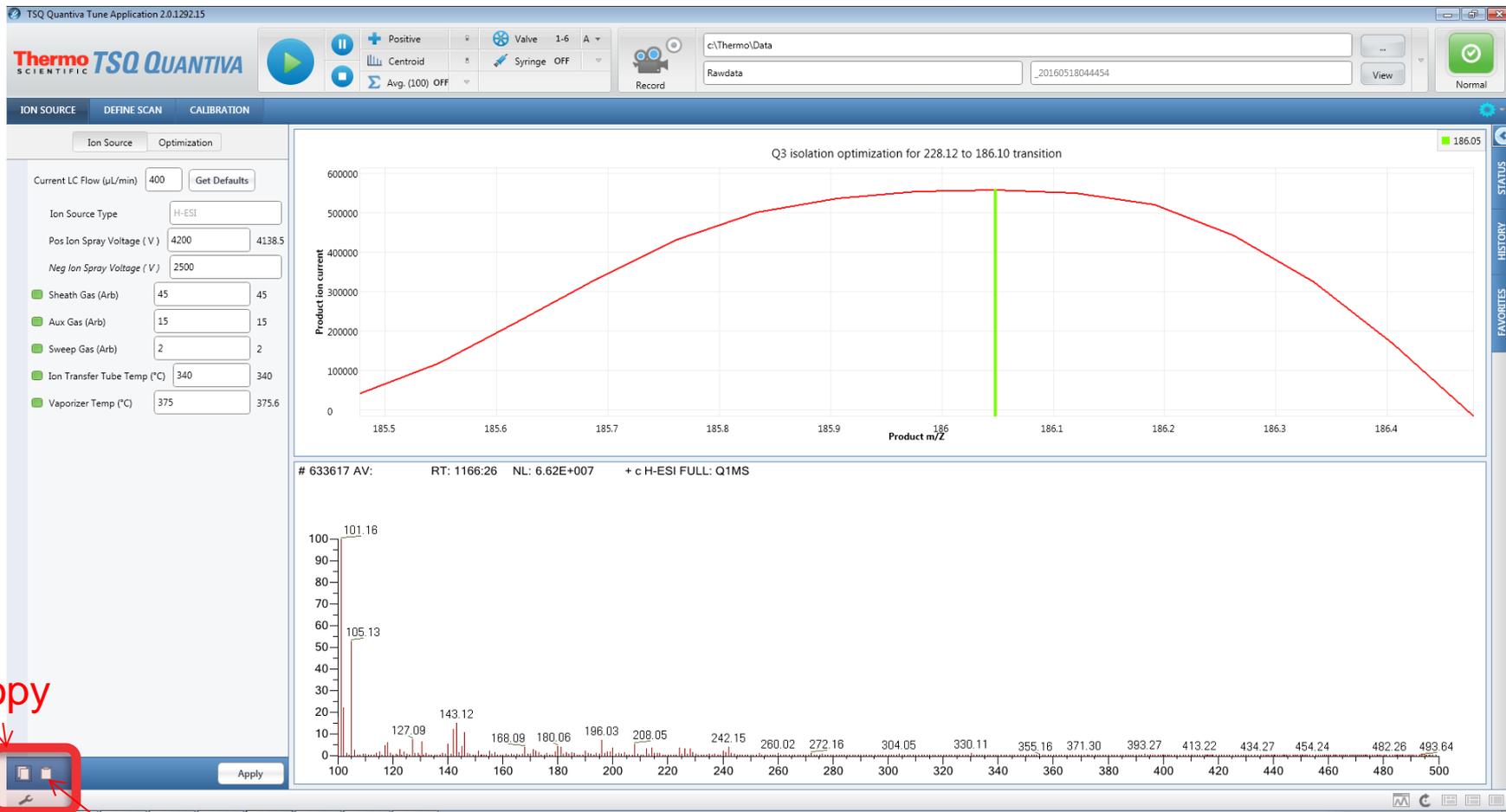


# Optimization of Source Conditions Using Automated Routines



# Copy Source Conditions

TSQ Tune Page



Copy

Paste

# Paste Source Conditions

## TSQ Instrument Method Editor

Method Editor Global Parameters Scan Parameters Summary

Method Timeline

| Method Duration (min) | # | 0.833 | 1.667 | 2.500 | 3.333 | 4.167 | 5 |
|-----------------------|---|-------|-------|-------|-------|-------|---|
| 5                     | 1 |       |       |       |       |       |   |

Global Parameters

Ion Source Properties

Ion Source Type: H-ESI

Spray Voltage: Static

Positive Ion (V): 4200.00

Negative Ion (V): 2500.00

Current LC Flow (µL/min): 0

Sheath Gas (Arb): 45

Aux Gas (Arb): 15

Sweep Gas (Arb): 2

Ion Transfer Tube Temp (°C): 340

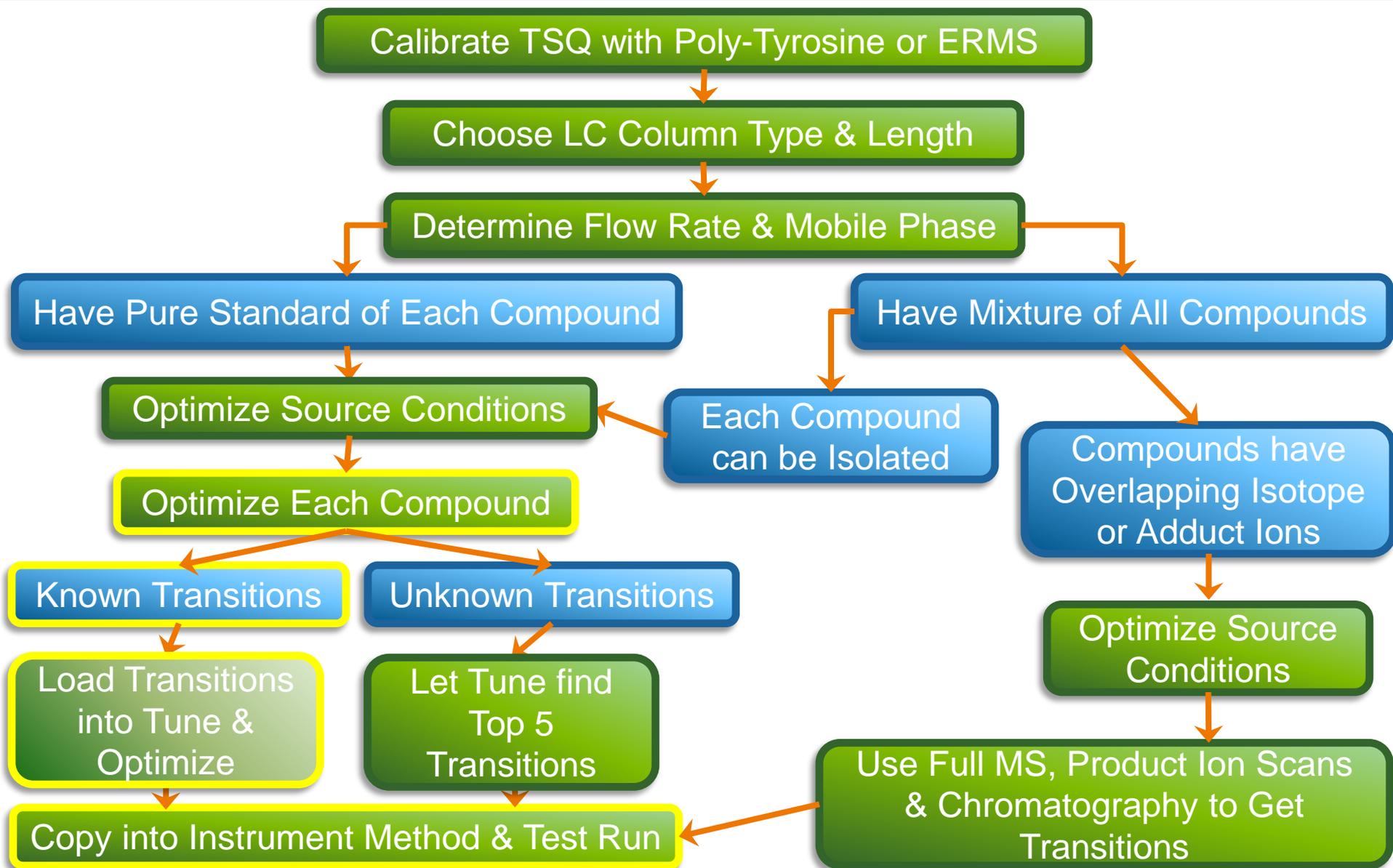
Vaporizer Temp (°C): 375

APPI Lamp: Not in Use

Copy Paste

Ready NOT SAVED

# Compound Optimization When the Transitions Are Known



# Compound Optimization of the Precursor

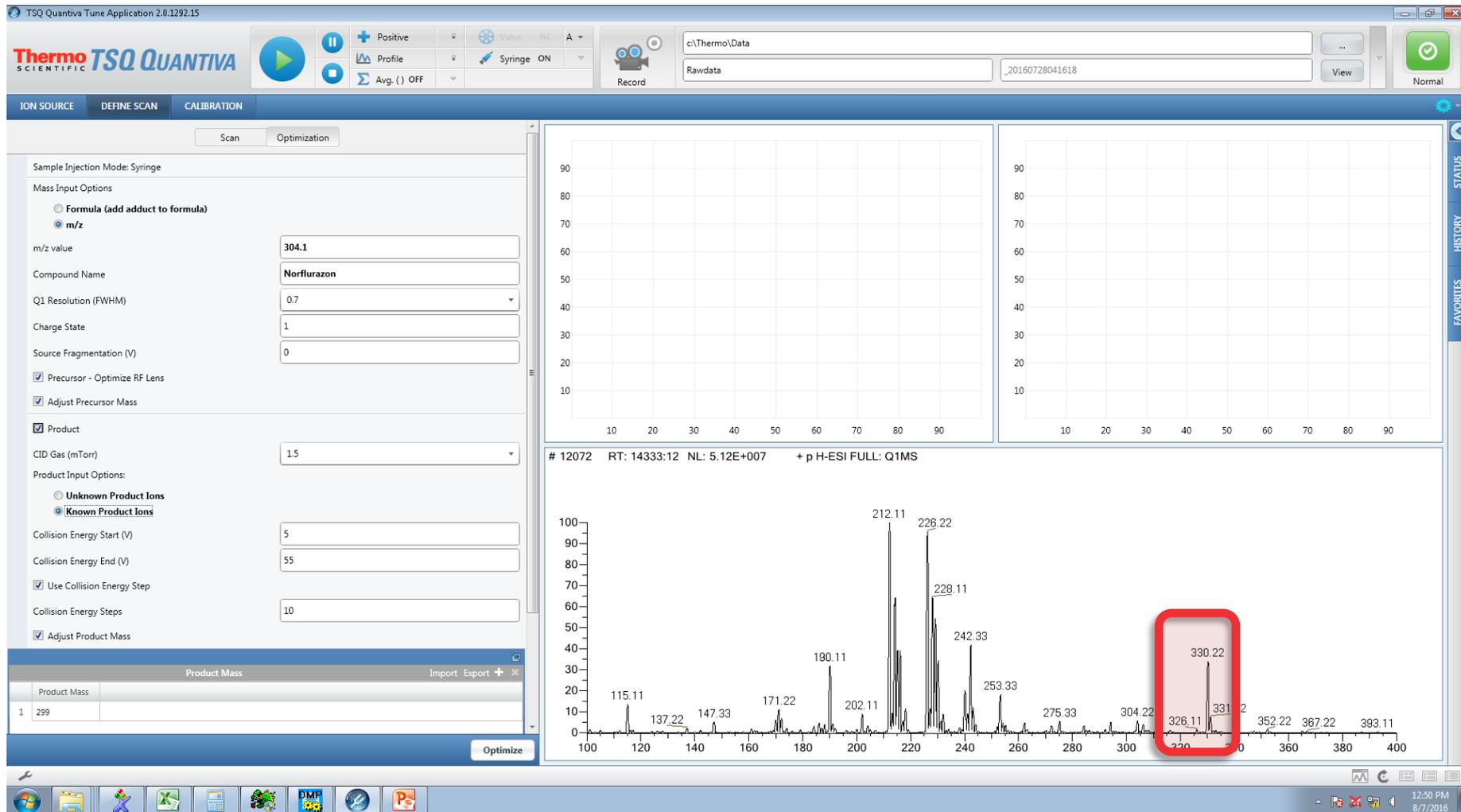
The screenshot displays the Thermo Scientific TSQ Quantiva software interface. The top navigation bar includes 'ION SOURCE', 'DEFINE SCAN', and 'CALIBRATION'. The 'Optimization' tab is selected and highlighted with a red box. The left sidebar contains configuration options for 'Sample Injection Mode: Syringe', 'Mass Input Options' (Formula and m/z), 'm/z value' (304.1), 'Compound Name' (Norflurazon), 'Q1 Resolution (FWHM)' (0.7), 'Charge State' (1), 'Source Fragmentation (V)' (0), 'Precursor - Optimize RF Lens' (checked), 'Adjust Precursor Mass' (checked), 'Product' (checked), 'CID Gas (mTorr)' (1.5), 'Product Input Options' (Unknown and Known Product Ions), 'Collision Energy Start (V)' (5), 'Collision Energy End (V)' (55), 'Use Collision Energy Step' (checked), 'Collision Energy Steps' (10), and 'Adjust Product Mass' (checked). A 'Product Mass' table is visible at the bottom left of the sidebar.

| Product Mass |     | Import | Export |
|--------------|-----|--------|--------|
| 1            | 299 |        |        |

The main window features two empty mass spectra plots at the top and a full mass spectrum plot at the bottom. The bottom plot is titled '# 12072 RT: 14333:12 NL: 5.12E+007 + p H-ESI FULL: Q1MS' and shows relative intensity versus m/z. Key peaks are labeled with their m/z values: 115.11, 137.22, 147.33, 171.22, 190.11, 202.11, 212.11, 226.22, 228.11, 242.33, 253.33, 275.33, 304.22, 326.11, 330.22, 331.22, 352.22, 367.22, and 393.11. The x-axis ranges from 100 to 400 m/z, and the y-axis ranges from 0 to 100 relative intensity.

Optimize Each Compound

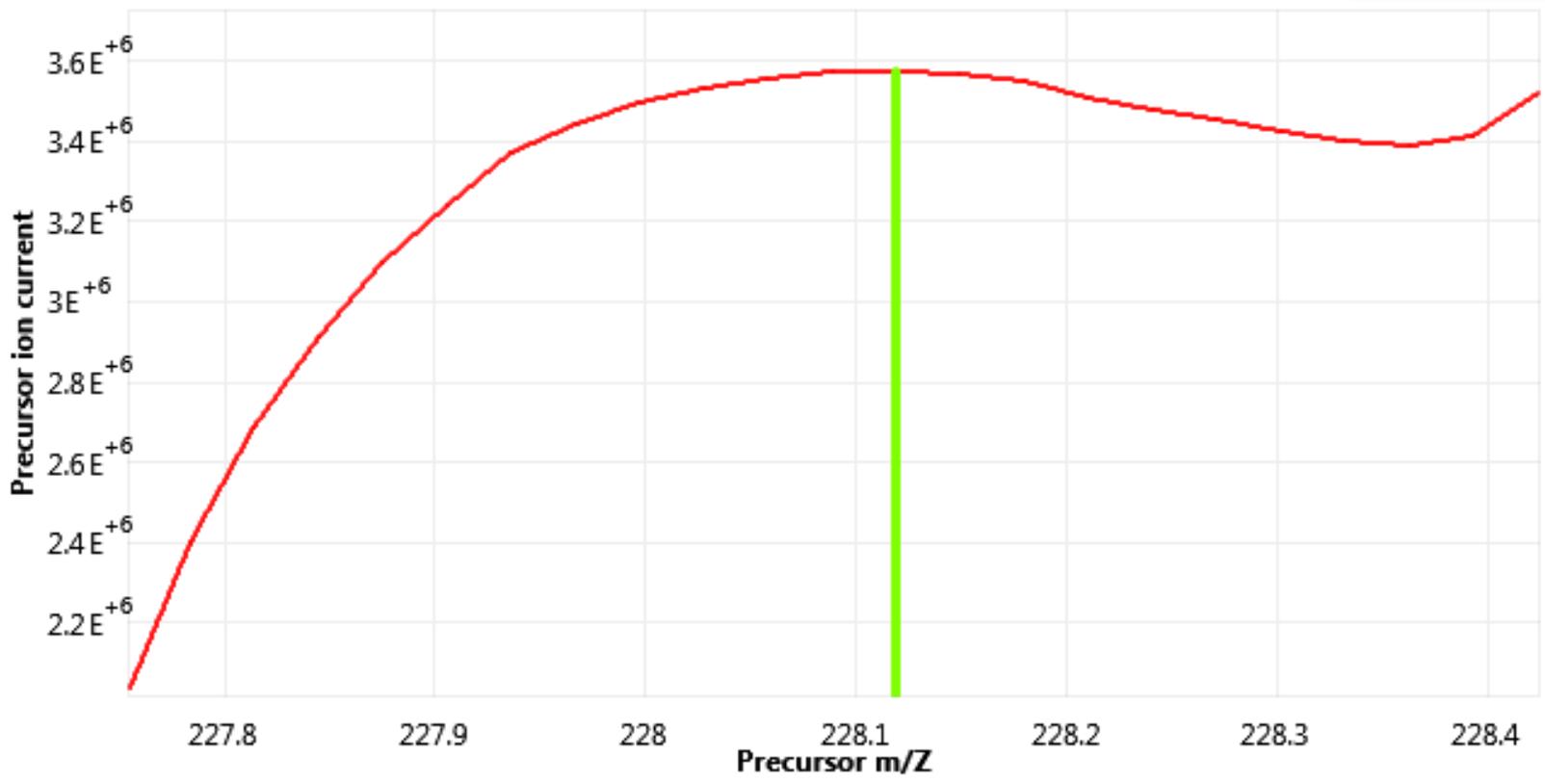
# Which Precursor Do We Use?



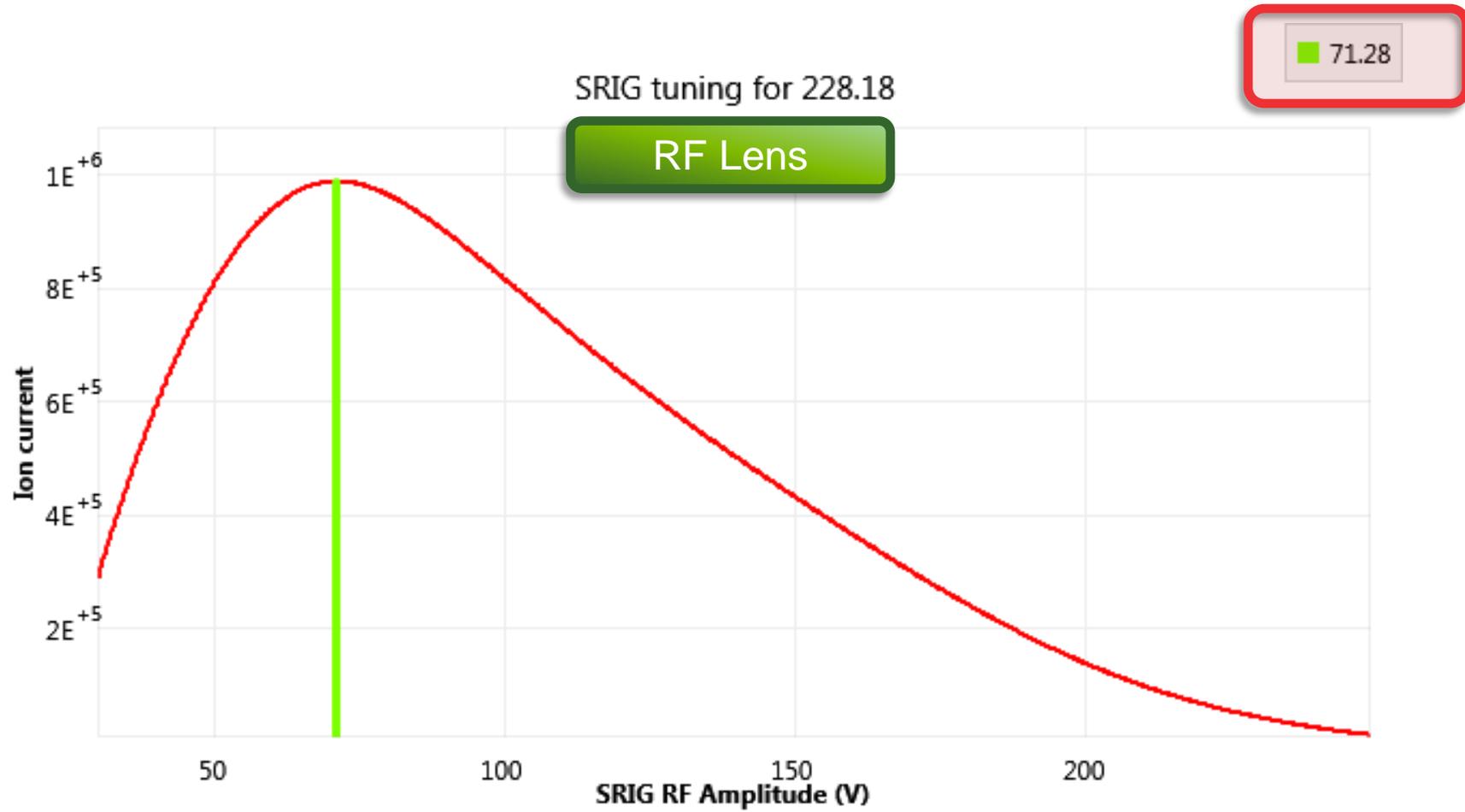
# Optimizes Precursor m/z

228.12

Precursor Mass Isolation for 228.18



# Optimizes RF Lens



# Using Product Ions from a TraceFinder Compound Database

The screenshot displays the Thermo TraceFinder Compound Database interface. The main window shows the details for Norflurazon (SRM C12H9ClF3N3O). The 'Target Peaks' section is expanded to show 'Peak 1' details, including precursor and product masses, polarity, adduct, and charge state. A red box highlights the 'Confirming Peaks' table, which lists precursor and product masses, collision energy, target ratio, and window type for two transitions.

| Precursor Mass | Product Mass | Collision Energy | Target Ratio(%) | Window Type | Window (+/- %) |
|----------------|--------------|------------------|-----------------|-------------|----------------|
| 304.080        | 160.000      | 32               |                 | Absolute    |                |
| 304.080        | 263.950      | 27               |                 | Absolute    |                |

Known Transitions

# Choosing Transitions from mzcloud.org

The screenshot displays the mzCloud web interface. The top navigation bar includes 'Home', 'About', 'Features', 'Partners', 'Contact', and 'Log in'. The main content area is divided into three columns: 'Reference Library', 'Spectral Tree', and 'Structure'.

**Reference Library:** A search filter for 'norflu' is applied. Results for 'norflu' are shown, including:

- Norfluoxetine (No: 849, Monoiso. Mass: 295.11840)
- Norfludiazepam (No: 2027, Monoiso. Mass: 288.04657)
- N-Desmethyflunitrazepam (No: 2426, Monoiso. Mass: 299.07062)
- Norflurazon (No: 2492, Monoiso. Mass: 303.03862)
- Norflvoxamine (No: 2493, Monoiso. Mass: 304.13986)

**Spectral Tree:** Shows 'Filtered' and 'Recalibrated' tabs. A 'Recalibrated Spectrum' plot is displayed with the title 'FTMS + APCI ms2 304.0459@hcd50.00 [50.00-314.05]'. The x-axis represents m/z from 0 to 300, and the y-axis represents relative intensity from 0 to 250,000,000. Key peaks are labeled with their m/z values: 87.99485, 160.03686, 193.06976, 264.03344, and 284.03967. The base peak is at m/z 284.03967. A red box highlights this spectrum.

**Structure:** Shows the chemical structure of Norflurazon, with the molecular formula  $C_{12}H_9ClF_3N_3O$ . The structure is a benzimidazole derivative with a chlorine atom at position 2, a trifluoromethyl group at position 5, and a 4-fluorophenyl group at position 1.

**Breakdown Curves:** A plot showing the relative intensity of precursor ions versus their m/z. A red box highlights this plot, which shows a prominent peak at m/z 284.03967, corresponding to the base peak in the main spectrum.

**Options:** The 'Options' panel on the right allows for selecting the ionization method (CID or HCD), the relative intensity (Rel or Abs), and the number of highest peaks to display (set to 5).

# Compound Optimization of Known Product Ions

The screenshot shows the Thermo TSQ Quantiva software interface. The 'CALIBRATION' tab is active, and the 'Optimization' sub-tab is highlighted with a red box. The 'Sample Injection Mode' is set to 'Syringe'. The 'Mass Input Options' section has 'Formula (add adduct to formula)' selected, and the 'm/z' value is set to 304.1. The 'Compound Name' is 'Norflurazon'. The 'Q1 Resolution (FWHM)' is 0.7, 'Charge State' is 1, and 'Source Fragmentation (V)' is 0. The 'Precursor - Optimize RF Lens' and 'Adjust Precursor Mass' checkboxes are checked. The 'Product' checkbox is also checked. The 'CID Gas (mTorr)' is set to 1.5. The 'Product Input Options' section has 'Known Product Ions' selected. The 'Collision Energy Start (V)' is 5, 'Collision Energy End (V)' is 55, 'Use Collision Energy Step' is checked, and 'Collision Energy Steps' is 10. The 'Adjust Product Mass' checkbox is checked. The 'Product Mass' table at the bottom left shows a single entry with a product mass of 299. The main panel displays a mass spectrum for scan #12072 at RT: 14333.12, NL: 5.12E+007, + p H-ESI FULL: Q1MS. The x-axis represents m/z from 100 to 400, and the y-axis represents relative intensity from 0 to 100. The base peak is at m/z 212.11. Other significant peaks are labeled at m/z 115.11, 137.22, 147.33, 171.22, 190.11, 202.11, 228.22, 228.11, 242.33, 253.33, 275.33, 304.22, 326.11, 330.22, 331.22, 352.22, 367.22, and 393.11.

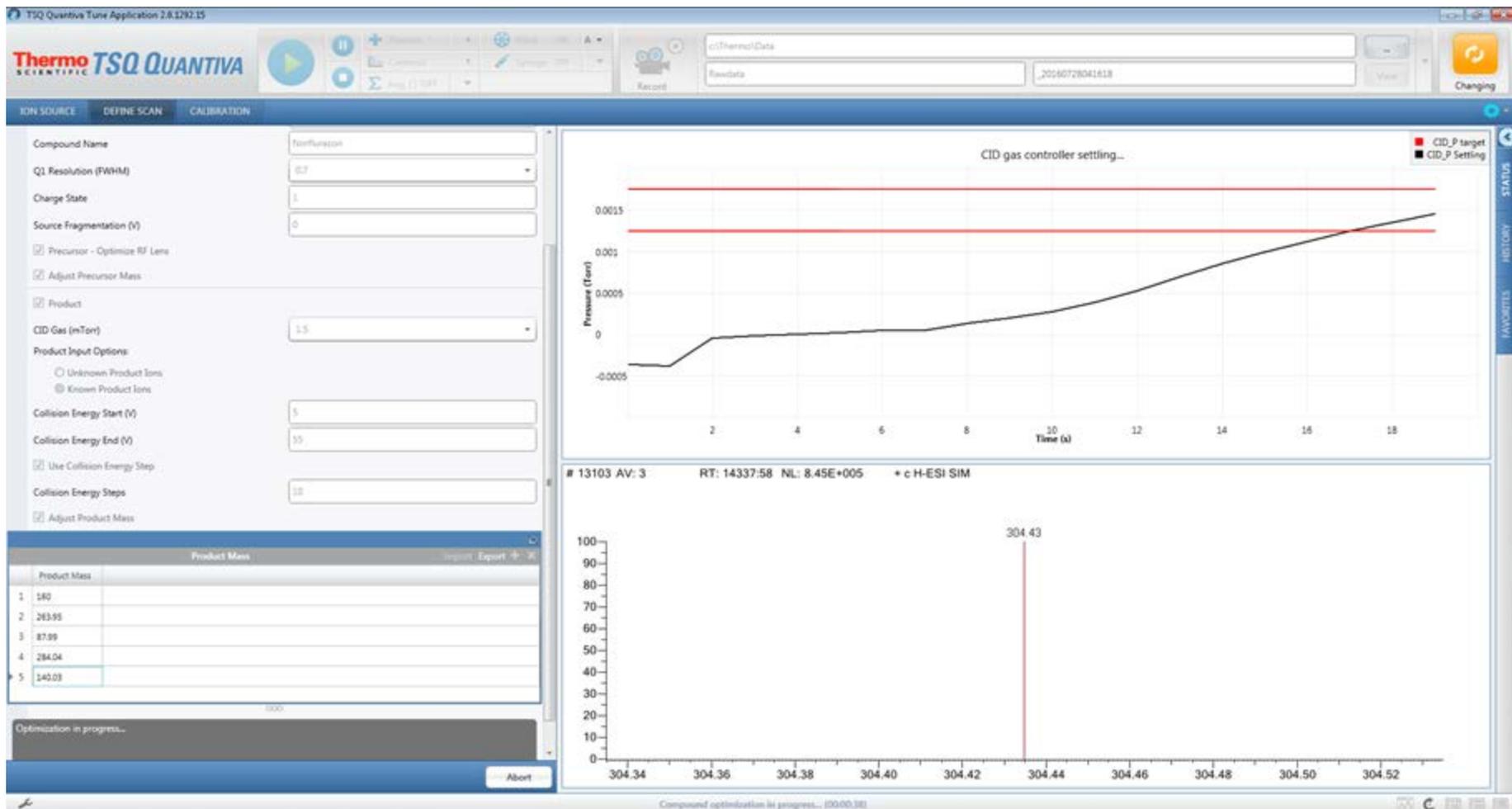
# Typing in the Known Product Ions

The screenshot displays the Thermo TSQ Quantiva software interface. The top menu bar includes 'ION SOURCE', 'DEFINE SCAN', and 'CALIBRATION'. The main window is divided into several sections:

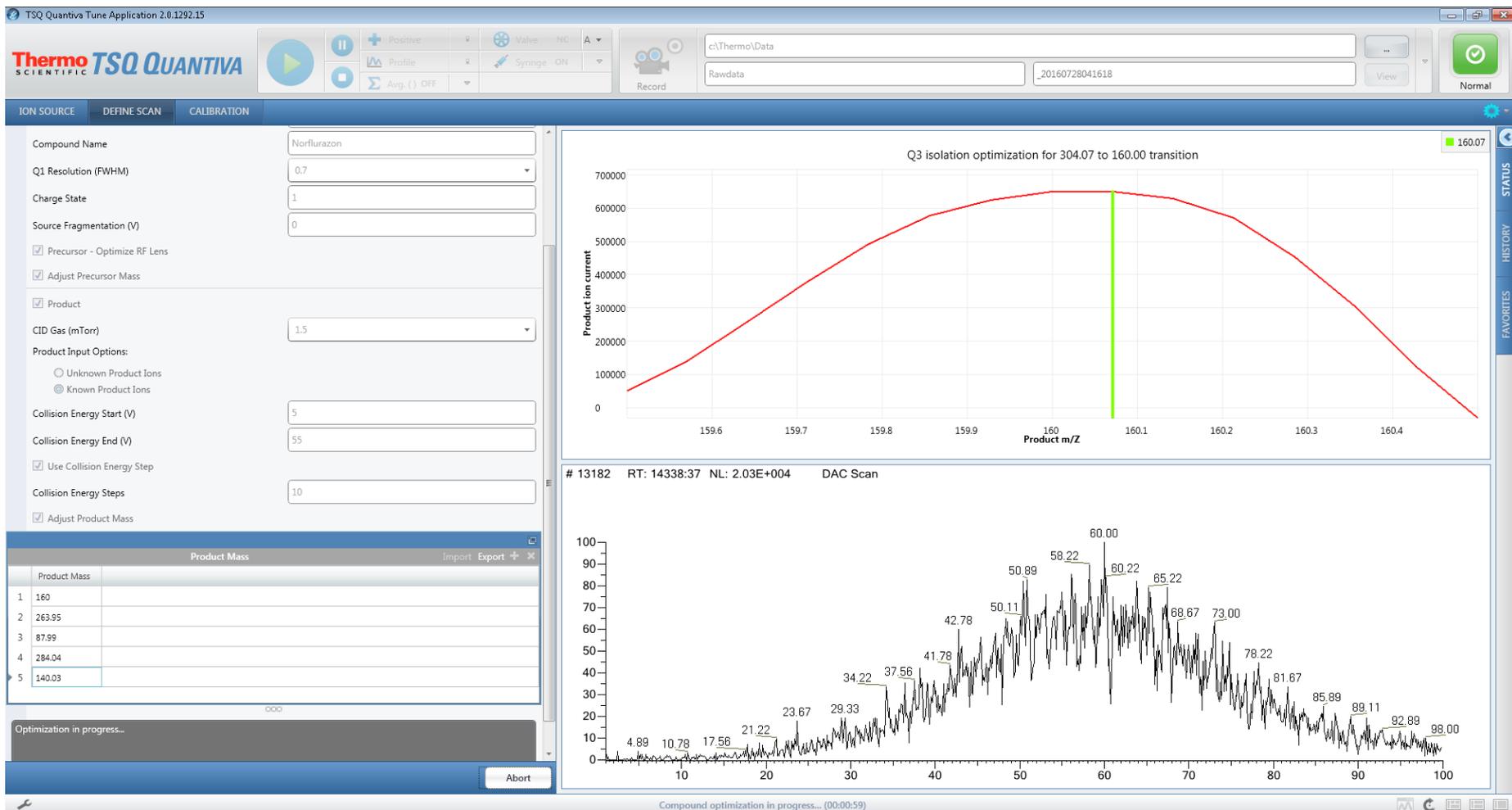
- Compound Information:** Compound Name: Norflurazon; Q1 Resolution (FWHM): 0.7; Charge State: 1; Source Fragmentation (V): 0.
- Product Input Options:**  Precursor - Optimize RF Lens;  Adjust Precursor Mass;  Product; CID Gas (mTorr): 1.5.
- Collision Energy Settings:** Collision Energy Start (V): 5; Collision Energy End (V): 55;  Use Collision Energy Step; Collision Energy Steps: 10;  Adjust Product Mass.
- Product Mass Table:** A table with 5 rows and 2 columns. The first row is highlighted with a red box. The data is as follows:

| Product Mass | Import | Export |
|--------------|--------|--------|
| 1            | 160    |        |
| 2            | 263.95 |        |
| 3            | 87.99  |        |
| 4            | 284.04 |        |
| 5            | 140.03 |        |
- Mass Spectrum Plot:** A plot showing relative intensity versus m/z. The x-axis ranges from 100 to 400, and the y-axis ranges from 0 to 100. The plot is titled '# 12879 RT: 14337.16 NL: 4.86E+007 + p H-ESI FULL: Q1MS'. Key peaks are labeled with their m/z values: 115.11, 117.00, 147.11, 171.00, 190.22, 191.11, 212.33, 228.22, 228.11, 242.22, 253.22, 262.22, 275.11, 304.22, 326.22, 330.11, 331.22, 352.22, 365.11, and 393.33.

# The Instrument Stabilizes the Collision gas in Q2



# The Product Ions are Optimized



# Breakdown Curves

TSQ Quantiva Tune Application 2.0.1292.15

Thermo **TSQ QUANTIVA** SCIENTIFIC

Record: c:\Thermo\Data Rawdata \_20160728041618 View Normal

ION SOURCE DEFINE SCAN CALIBRATION

Scan Optimization

Sample Injection Mode: Syringe

Mass Input Options

- Formula (add adduct to formula)
- m/z

m/z value: 304.1

Compound Name: Norflurazon

Q1 Resolution (FWHM): 0.7

Charge State: 1

Source Fragmentation (V): 0

Precursor - Optimize RF Lens

Adjust Precursor Mass

Product

CID Gas (mTorr): 1.5

Product Input Options

- Unknown Product Ions
- Known Product Ions

Collision Energy Start (V): 5

Collision Energy End (V): 55

Use Collision Energy Step

Collision Energy Steps: 10

Adjust Product Mass

Exclude Loss Masses: 18,17,15 [Edit Mass List](#)

top N: 5

Low Mass Exclusion: 10

Abort

Breakdown Curve for 304.07 to 284.00 transition

Multiple collision energy product scan for m/z=304.07.

# 14175 AV: 3 RT: 14344:16 NL: 3.46E+006 + c H-ESI SRM: 304.0696

Compound optimization in progress... (00:01:13)

1:01 PM 8/7/2016

# Copying the Transitions to the Instrument Method

TSQ Quantiva Tune Application 2.8.120.15

Thermo **TSQ QUANTIVA** SCIENTIFIC

ION SOURCE DETRME SCAN CALIBRATION

Product Input Options

- Unknown Product Ions
- Known Product Ions

Collision Energy Start (V): 5

Collision Energy End (V): 35

Use Collision Energy Step

Collision Energy Steps: 10

Adjust Product Mass

Product Mass

| Product Mass |
|--------------|
| 1 160        |
| 2 263.05     |
| 3 87.99      |
| 4 284.04     |
| 5 140.03     |

Optimization is progress...

Optimization Results

| Compound       | Precursor (m/z) | RF Lens (V) | Product (m/z) | Collision Energy (V) | Intensity  | Source Fragmentation |
|----------------|-----------------|-------------|---------------|----------------------|------------|----------------------|
| 1 Non-Function | 304.07          | 120.584     | 160.07        | 54.219               | 649923.584 | 0                    |
| 2 Non-Function | 304.07          | 120.584     | 264.02        | 29.062               | 182077.557 | 0                    |
| 3 Non-Function | 304.07          | 120.584     | 87.99         | 46.961               | 551644.227 | 0                    |
| 4 Non-Function | 304.07          | 120.584     | 284.04        | 24.713               | 400225.211 | 0                    |
| 5 Non-Function | 304.07          | 120.584     | 140.03        | 59.225               | 529631.62  | 0                    |

Q3 isolation optimization for 304.07 to 140.03 transition

# 13678 RT: 14340.56 NL: 4.62E+006 + c H-ESI SRM: 304.0690

# Drag and Drop SRM Scan

## TSQ Instrument Method Editor

Method Editor

Global Parameters Scan Parameters Summary

Method Timeline

Method Duration (min) 5

Experiment 1

| Compound | Retention Time (min) | RT Window (min) | Polarity | Precursor (m/z) | Product (m/z) | Collision Energy (V) |
|----------|----------------------|-----------------|----------|-----------------|---------------|----------------------|
| name     | 1                    | 1               | Positive | 200             | 100           | 0                    |

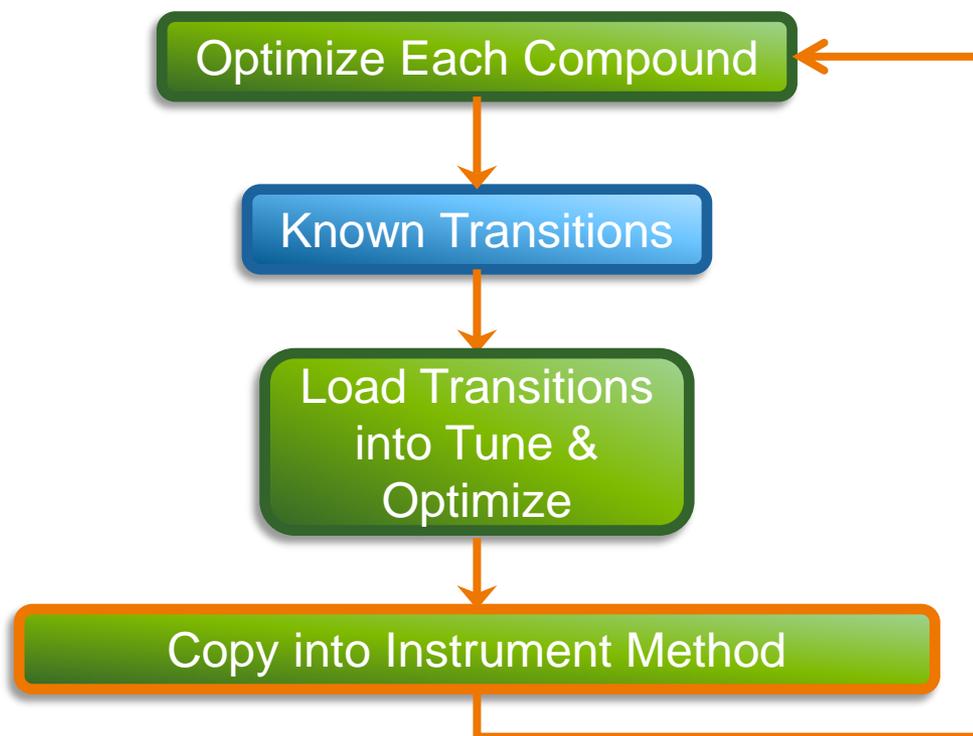
SRM Properties

- Use Cycle Time
- Use Calibrated RF Lens
- Q1 Resolution (FWHM) 0.7
- Q3 Resolution (FWHM) 0.7
- CID Gas (mTorr) 1.5
- Source Fragmentation (V) 0
- Chrom Filter (sec) 3
- Display Retention Time

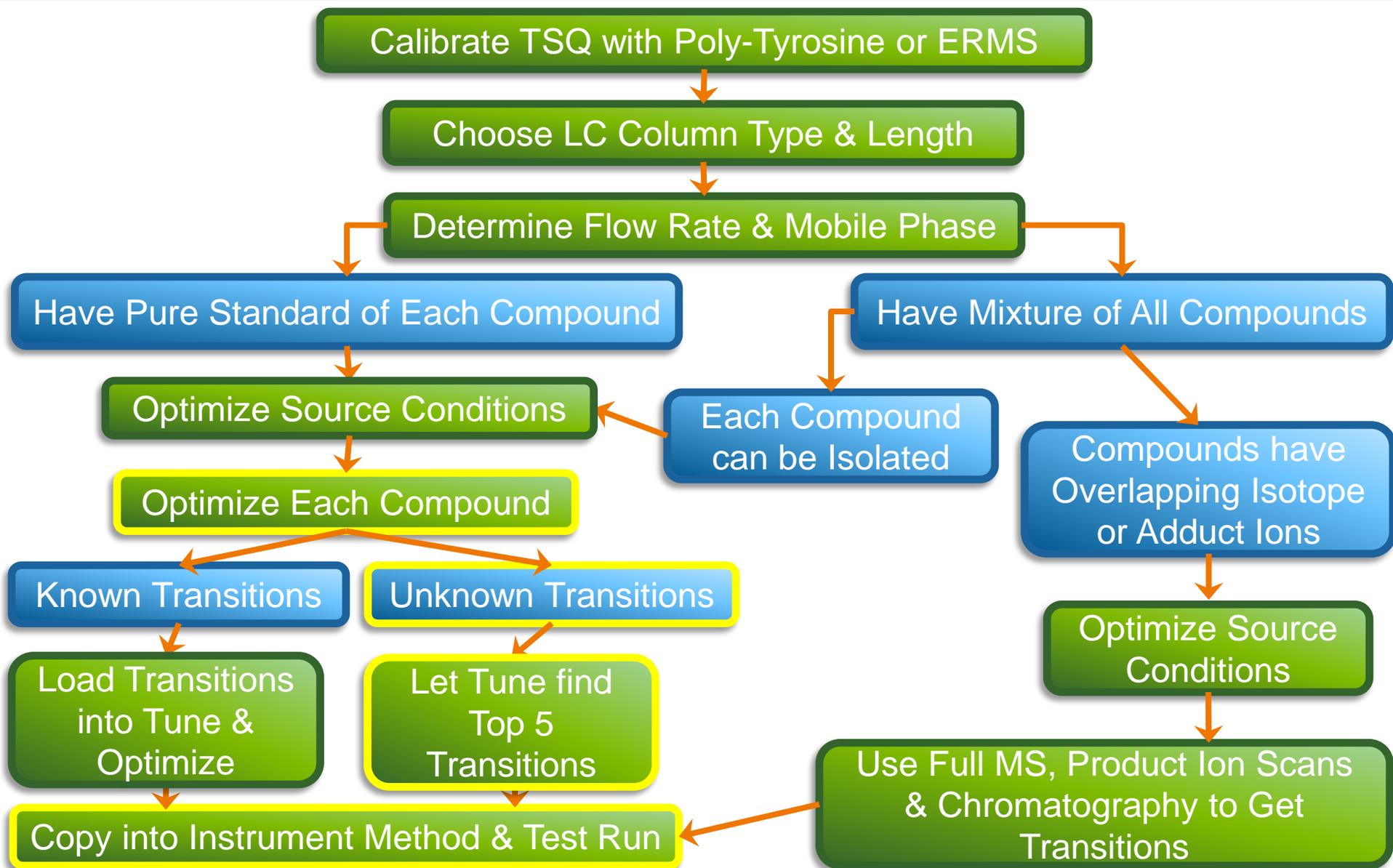
Copy Experiment Time

Uncheck "Use Calibrated RF Lens" box

# Repeat for Each Compound



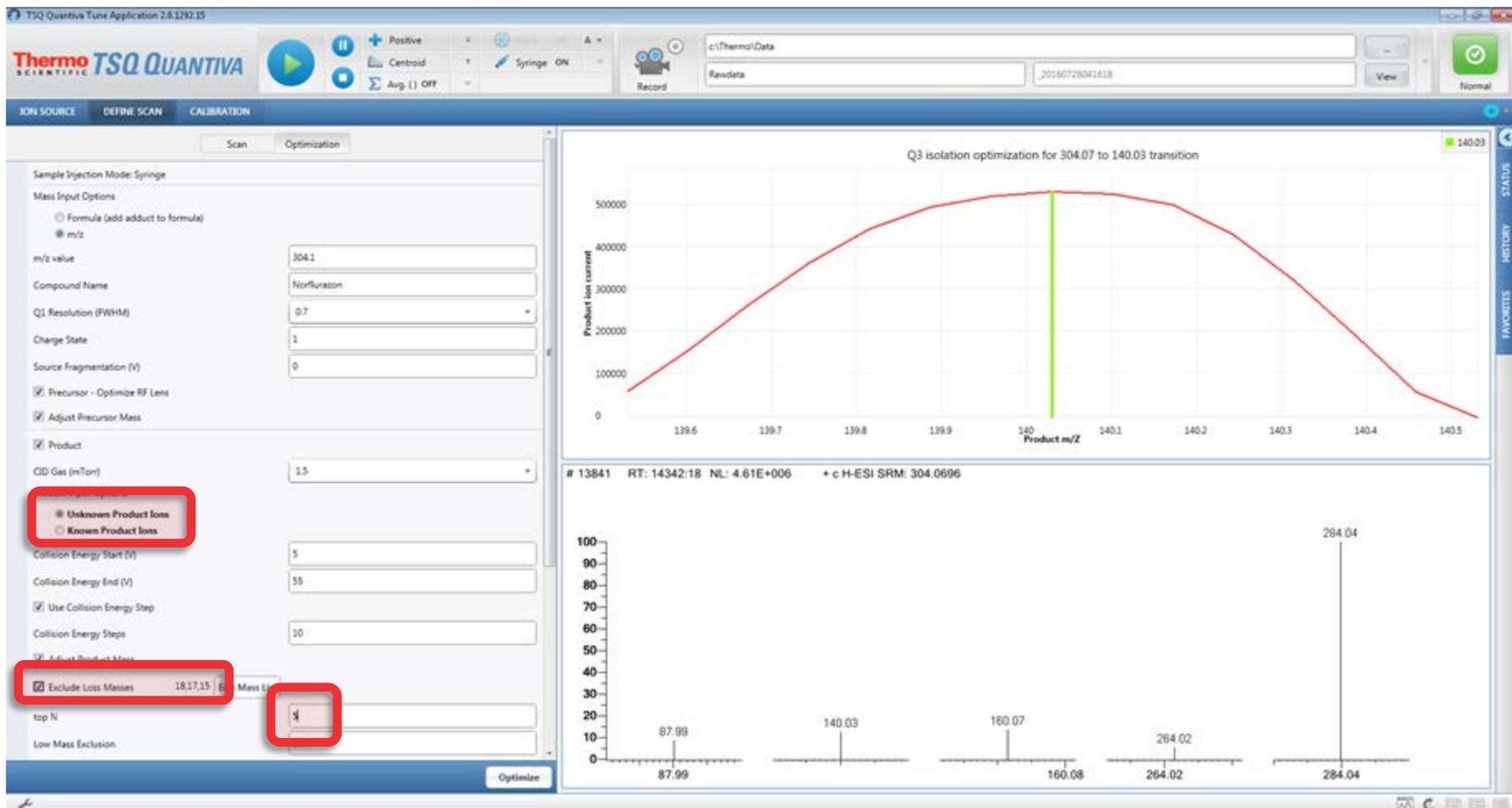
# Compound Optimization When the Transitions Aren't Known



# Thoughts on Choosing Unknown Product Ions...

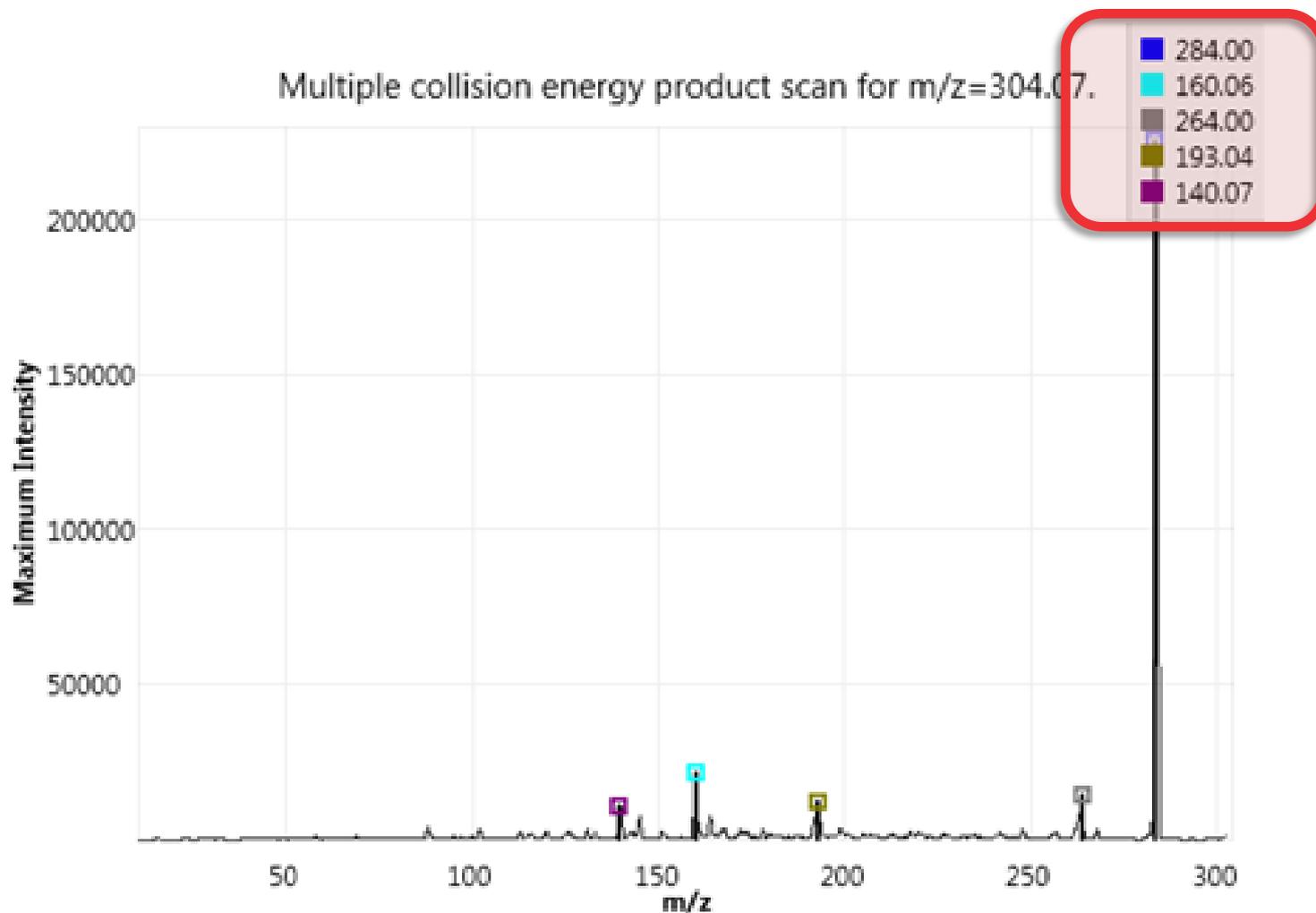
- Try to avoid choosing common losses such as ammonia and water as these add little specificity to the SRM transition
- Be aware of using high collision energies and generating fragment ions that are very small and common to a large number of compounds
- In general its desirable to choose five product 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

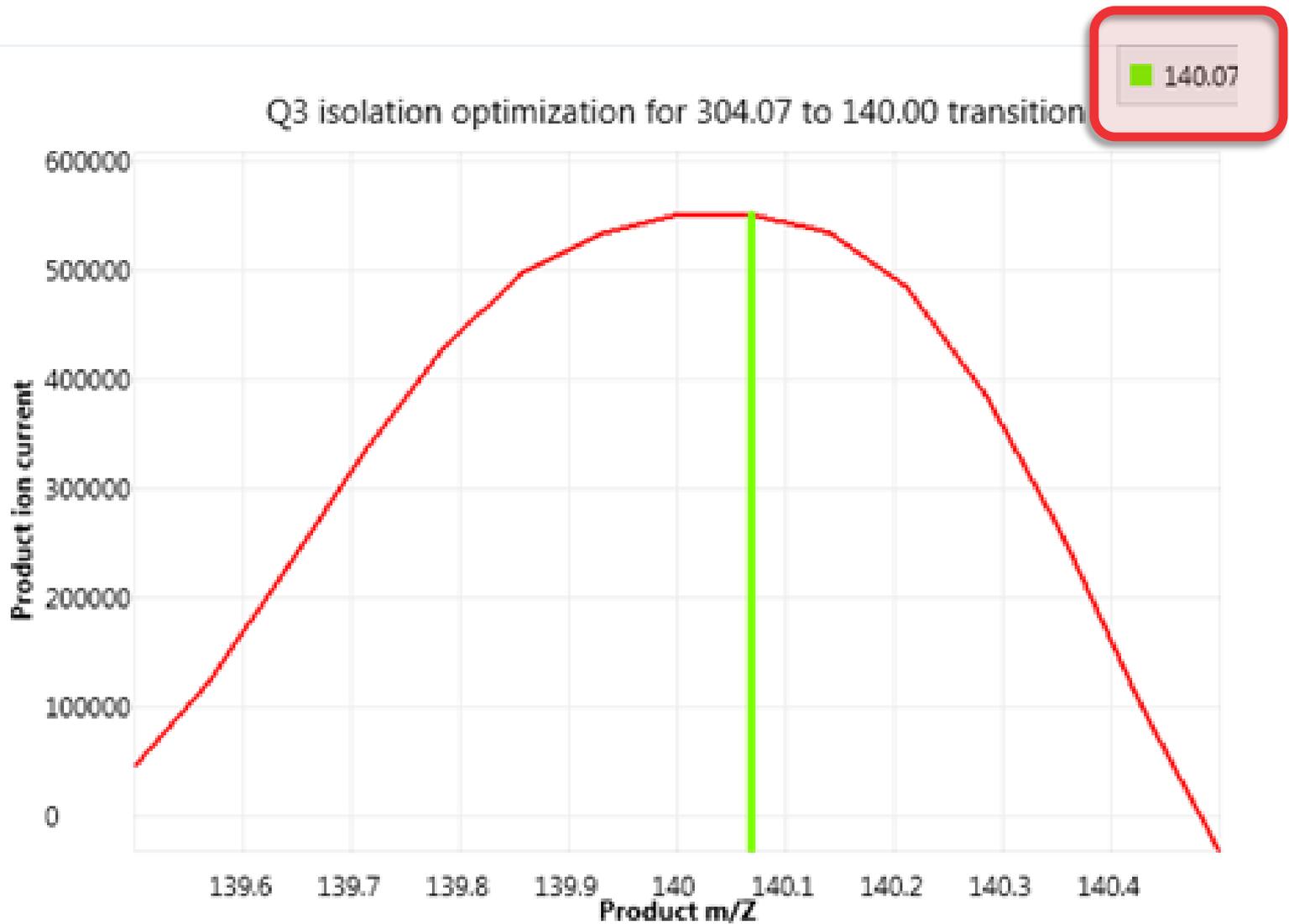


Unknown Transitions

# The Instrument Finds the Best Product Ions



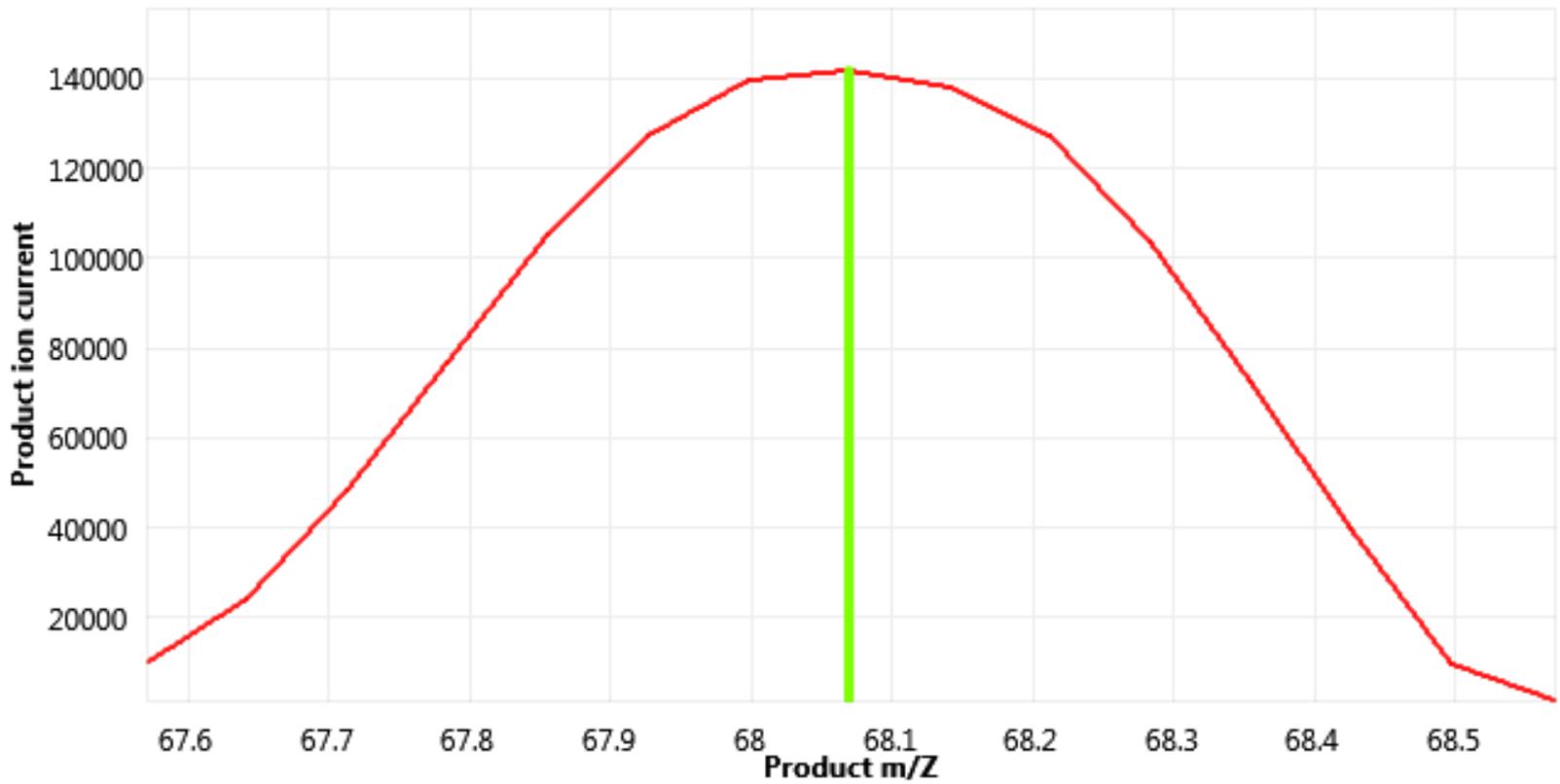
# Creates Breakdown Curve



# Optimizes Q3 m/z

68.07

Q3 isolation optimization for 228.12 to 68.32 transition



# Compound Optimization Results

| Optimization Results |              |                 |             |               |                      |             | Import Export + X    |
|----------------------|--------------|-----------------|-------------|---------------|----------------------|-------------|----------------------|
|                      | Compound     | Precursor (m/z) | RF Lens (V) | Product (m/z) | Collision Energy (V) | Intensity   | Source Fragmentation |
| 1                    | Norflurazone | 304.07          | 110.584     | 160.071       | 34.219               | 649623.394  | 0                    |
| 2                    | Norflurazone | 304.07          | 110.584     | 264.001       | 29.062               | 182077.557  | 0                    |
| 3                    | Norflurazone | 304.07          | 110.584     | 87.99         | 46.961               | 353444.207  | 0                    |
| 4                    | Norflurazone | 304.07          | 110.584     | 284.04        | 34.713               | 4002125.111 | 0                    |
| 5                    | Norflurazone | 304.07          | 110.584     | 140.03        | 39.225               | 529835.62   | 0                    |

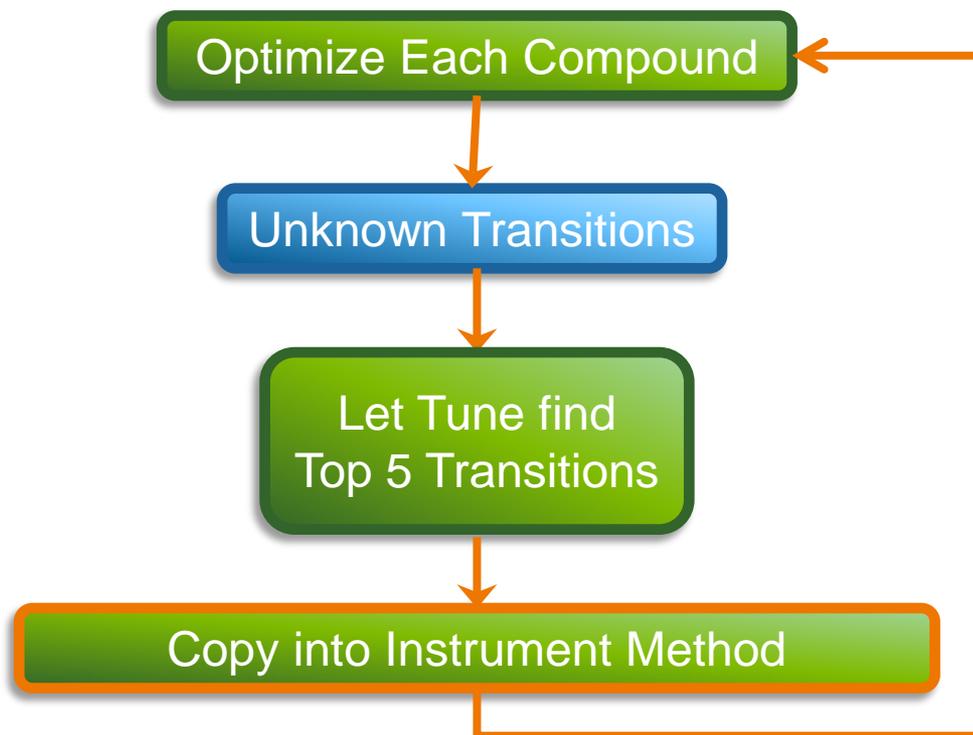
# Drag and Drop SRM Scan

The screenshot displays the 'Method Editor' interface for 'Experiment 1'. The 'Scan Parameters' tab is active, showing a 'Method Timeline' at the top with a duration of 5 minutes. Below the timeline, the 'Scan Types' panel on the left lists various scan types, with 'SRM' highlighted by a red box. The 'SRM Table' in the center contains the following data:

| Compound | Retention Time (min) | RT Window (min) | Polarity | Precursor (m/z) | Product (m/z) | Collision Energy (V) |
|----------|----------------------|-----------------|----------|-----------------|---------------|----------------------|
| 1 name   | 1                    | 1               | Positive | 200             | 100           | 0                    |

On the right, the 'SRM Properties' panel includes settings for 'Use Cycle Time' (checked), 'Cycle Time (sec)' (1), 'Use Calibrated RF Lens' (checked), 'Q1 Resolution (FWHM)' (0.7), 'Q3 Resolution (FWHM)' (0.7), 'CID Gas (mTorr)' (1.5), 'Source Fragmentation (V)' (0), 'Chrom Filter (sec)' (3), and 'Display Retention Time' (checked). A 'Copy Experiment Time' button is located at the bottom of this panel.

# Repeat for Each Compound

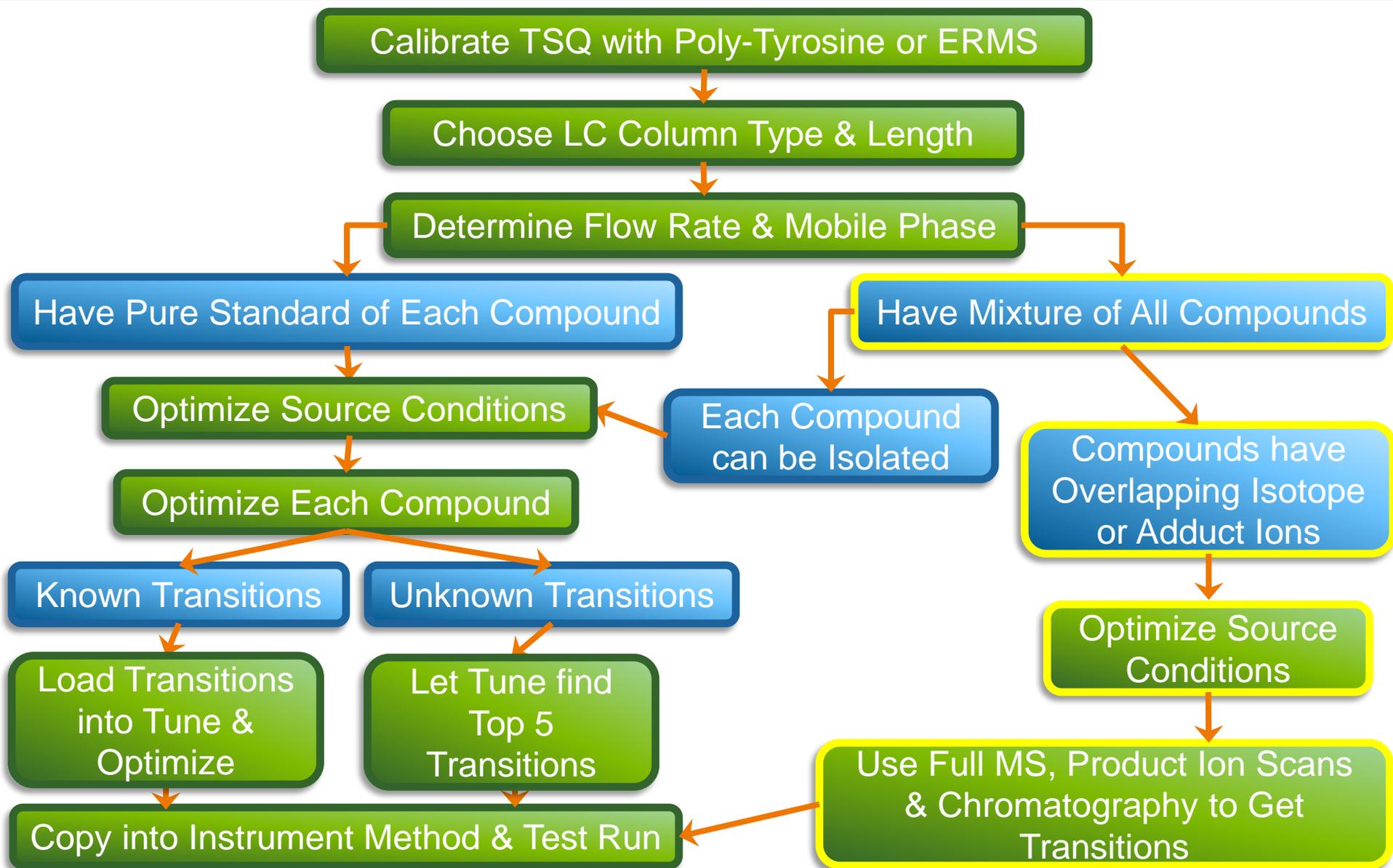


# Import the SRM Table

The screenshot displays the 'Method Editor' interface for 'Experiment 1'. The 'SRM Table' is the central focus, listing 25 compounds with their retention times, precursors, and collision energies. The 'Import' button in the top right of the table area is highlighted with a red box. The 'SRM Properties' panel on the right shows various parameters like 'Use Cycle Time', 'Cycle Time (sec)', and 'Q1 Resolution (FWHM)'. The 'Method Timeline' at the top shows a duration of 5 minutes.

| #  | Compound   | Retention Time (min) | RT Window (min) | Polarity | Precursor (m/z) | Product (m/z) | Collision Energy (V) |
|----|------------|----------------------|-----------------|----------|-----------------|---------------|----------------------|
| 1  | Alachlor   | 2.5                  | 5               | Positive | 270.195         | 162.155       | 21                   |
| 2  | Alachlor   | 2.5                  | 5               | Positive | 270.195         | 238.073       | 13                   |
| 3  | Ametryn    | 2.5                  | 5               | Positive | 228.18          | 68.32         | 38                   |
| 4  | Ametryn    | 2.5                  | 5               | Positive | 228.18          | 186.102       | 21                   |
| 5  | Atraton    | 2.5                  | 5               | Positive | 212.25          | 100.199       | 30                   |
| 6  | Atraton    | 2.5                  | 5               | Positive | 212.25          | 170.137       | 20                   |
| 7  | Atrazine   | 2.5                  | 5               | Positive | 216.1           | 104.164       | 31                   |
| 8  | Atrazine   | 2.5                  | 5               | Positive | 216.1           | 174.063       | 20                   |
| 9  | Bromacil   | 2.5                  | 5               | Positive | 261.095         | 187.872       | 30                   |
| 10 | Bromacil   | 2.5                  | 5               | Positive | 261.095         | 204.989       | 17                   |
| 11 | Butachlor  | 2.5                  | 5               | Positive | 312.17          | 162.195       | 26                   |
| 12 | Butachlor  | 2.5                  | 5               | Positive | 312.17          | 238.136       | 10                   |
| 13 | Butylate   | 2.5                  | 5               | Positive | 218.21          | 41.518        | 27                   |
| 14 | Butylate   | 2.5                  | 5               | Positive | 218.21          | 57.428        | 19                   |
| 15 | Cyanazine  | 2.5                  | 5               | Positive | 241.15          | 104.135       | 32                   |
| 16 | Cyanazine  | 2.5                  | 5               | Positive | 241.15          | 214.103       | 19                   |
| 17 | Cycloate   | 2.5                  | 5               | Positive | 216.215         | 55.422        | 29                   |
| 18 | Cycloate   | 2.5                  | 5               | Positive | 216.215         | 83.301        | 19                   |
| 19 | Diphenamid | 2.5                  | 5               | Positive | 240.215         | 134.16        | 24                   |
| 20 | Diphenamid | 2.5                  | 5               | Positive | 240.215         | 167.134       | 25                   |
| 21 | EPFC       | 2.5                  | 5               | Positive | 190.105         | 43.693        | 21                   |
| 22 | EPFC       | 2.5                  | 5               | Positive | 190.105         | 86.171        | 16                   |
| 23 | Fluridone  | 2.5                  | 5               | Positive | 330.085         | 290.126       | 34                   |
| 24 | Fluridone  | 2.5                  | 5               | Positive | 330.085         | 310.117       | 31                   |
| 25 | Hexazinone | 2.5                  | 5               | Positive | 253.245         | 71.332        | 32                   |

# Using an LCMS Method to Find Missing Compounds



# Using Product Ions Scans to Find Missing SRMs

ProductionScans - Thermo Xcalibur Instrument Setup

File TSQ Quantiva Help

### Method Editor

Global Parameters Scan Parameters Summary

Method Timeline

Method Duration (min): 3.5

Scan Points: 0.583, 1.167, 1.750, 2.333, 2.917, 3.500

Experiment 1

#### Scan Types

- SRM
- Full Scan Q1
- Full Scan Q3
- Product Ion Scan
- Precursor Ion Scan
- Neutral Loss Scan
- SIM Q1
- SIM Q3
- QED
- CO

#### Product Ion Scan Table

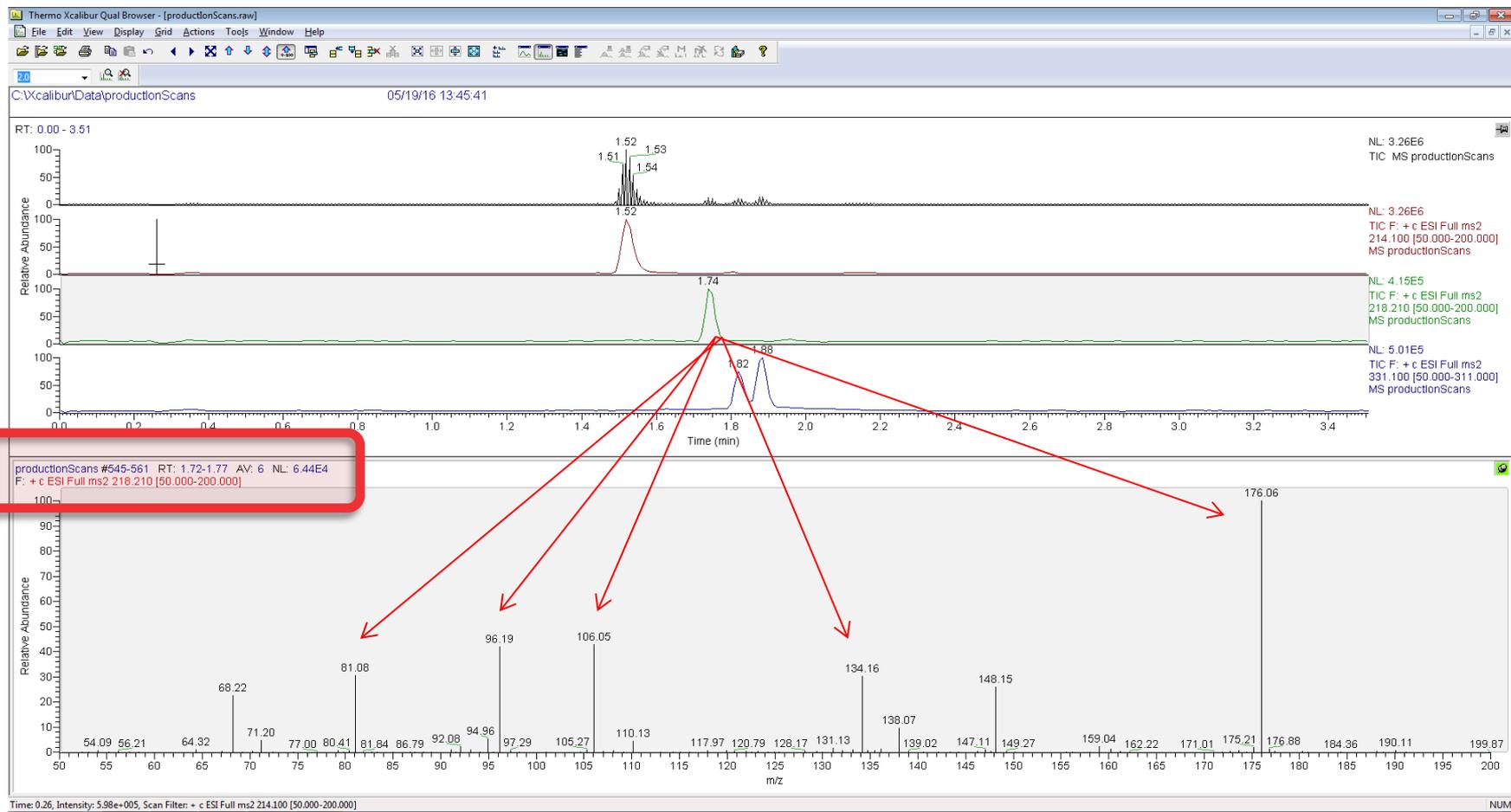
|   | Precursor (m/z) | Polarity | Scan Range (m/z) |
|---|-----------------|----------|------------------|
| 1 | 214.1           | Positive | 50-200           |
| 2 | 218.21          | Positive | 50-200           |
| 3 | 331.1           | Positive | 50-311           |

#### Product Ion Scan Properties

- Scan Rate (Da/sec): 1000
- Use Collision Energy Ramp (CER):
- Collision Energy (V): 25
- Use Calibrated RF Lens:
- Q1 Resolution (FWHM): 0.7
- Q3 Resolution (FWHM): 0.7
- CID Gas (mTorr): 1.5
- Source Fragmentation (V): 0
- Chrom Filter (sec): 3

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

# Finding Ions to Add



# Adding the New Compounds into the Method

Method Editor - Global Parameters | Scan Parameters | Summary

Method Timeline

Method Duration (min): 5

Experiment 1

| Compound      | Retention Time (min) | RT Window (min) | Polarity | Precursor (m/z) | Product (m/z) | Collision Energy (V) |
|---------------|----------------------|-----------------|----------|-----------------|---------------|----------------------|
| 1 Alachlor    | 2.5                  | 5               | Positive | 270.195         | 162.155       | 21                   |
| 2 Alachlor    | 2.5                  | 5               | Positive | 270.195         | 238.073       | 13                   |
| 3 Ametryn     | 2.5                  | 5               | Positive | 228.18          | 68.32         | 38                   |
| 4 Ametryn     | 2.5                  | 5               | Positive | 228.18          | 186.102       | 21                   |
| 5 Atraton     | 2.5                  | 5               | Positive | 212.25          | 100.199       | 30                   |
| 6 Atraton     | 2.5                  | 5               | Positive | 212.25          | 170.137       | 20                   |
| 7 Atrazine    | 2.5                  | 5               | Positive | 216.1           | 104.164       | 31                   |
| 8 Atrazine    | 2.5                  | 5               | Positive | 216.1           | 174.063       | 20                   |
| 9 Bromacil    | 2.5                  | 5               | Positive | 261.095         | 187.872       | 30                   |
| 10 Bromacil   | 2.5                  | 5               | Positive | 261.095         | 204.989       | 17                   |
| 11 Butachlor  | 2.5                  | 5               | Positive | 312.17          | 162.195       | 26                   |
| 12 Butachlor  | 2.5                  | 5               | Positive | 312.17          | 238.136       | 10                   |
| 13 Butylate   | 2.5                  | 5               | Positive | 218.21          | 41.518        | 27                   |
| 14 Butylate   | 2.5                  | 5               | Positive | 218.21          | 57.428        | 19                   |
| 15 Cyanazine  | 2.5                  | 5               | Positive | 241.15          | 104.135       | 32                   |
| 16 Cyanazine  | 2.5                  | 5               | Positive | 241.15          | 214.103       | 19                   |
| 17 Cycloate   | 2.5                  | 5               | Positive | 216.215         | 55.422        | 29                   |
| 18 Cycloate   | 2.5                  | 5               | Positive | 216.215         | 83.301        | 19                   |
| 19 Diphenamid | 2.5                  | 5               | Positive | 240.215         | 134.16        | 24                   |
| 20 Diphenamid | 2.5                  | 5               | Positive | 240.215         | 167.134       | 25                   |
| 21 EPTC       | 2.5                  | 5               | Positive | 190.105         | 43.693        | 21                   |
| 22 EPTC       | 2.5                  | 5               | Positive | 190.105         | 86.171        | 16                   |
| 23 Fluridone  | 2.5                  | 5               | Positive | 330.085         | 290.126       | 34                   |
| 24 Fluridone  | 2.5                  | 5               | Positive | 330.085         | 310.117       | 31                   |
| 25 Hexazinone | 2.5                  | 5               | Positive | 253.245         | 71.332        | 32                   |

SRM Properties

- Use Calibrated RF Lens
- Q1 Resolution (FWHM): 0.7
- Q3 Resolution (FWHM): 0.7
- CID Gas (mTorr): 1.5
- Source Fragmentation (V): 0
- Chrom Filter (sec): 3
- Display Retention Time

Copy Experiment Time

# SRM Properties – Cycle Time

SRM Properties

Use Cycle Time

Cycle Time (sec)

Use Calibrated RF Lens

Q1 Resolution (FWHM)  +

Q3 Resolution (FWHM)  +

CID Gas (mTon)  +

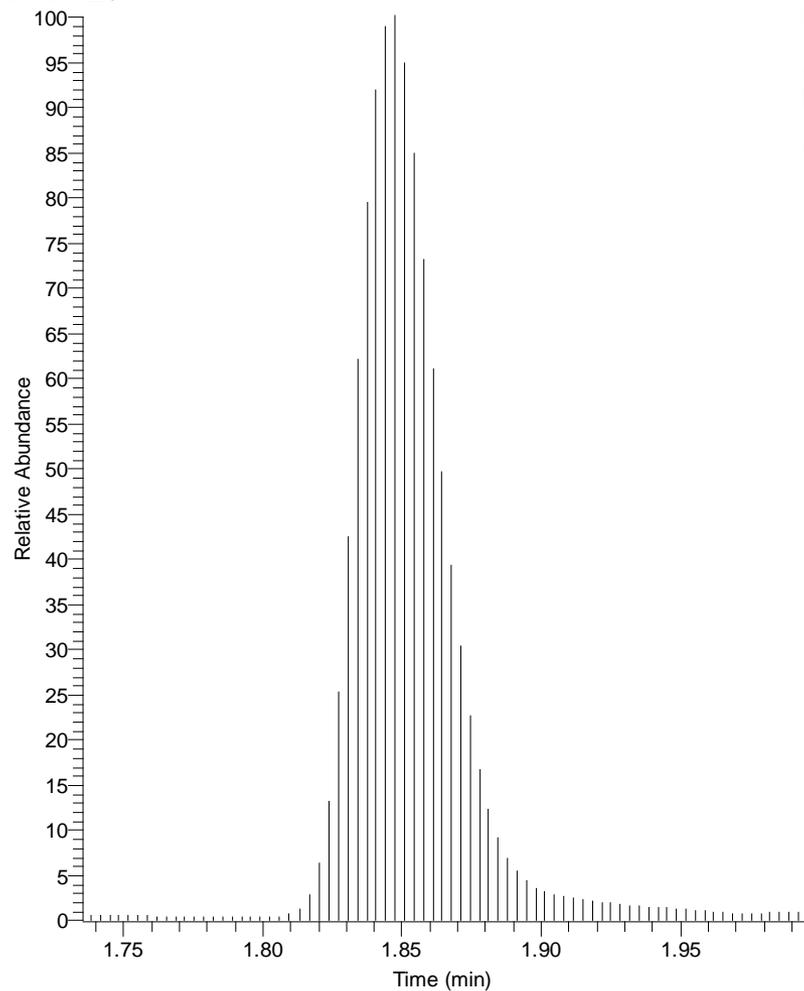
Source Fragmentation (V)

Chrom Filter (sec)

Display Retention Time

Copy Experiment Time

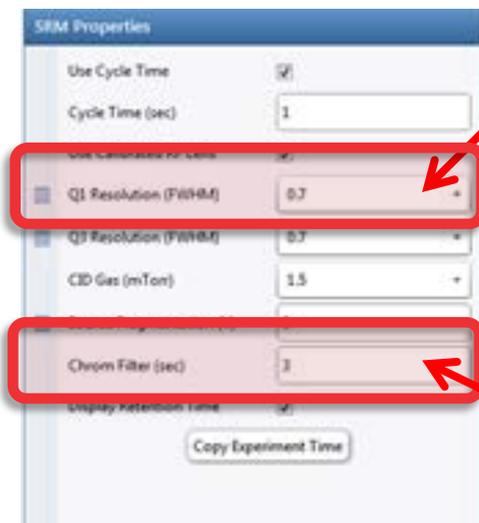
RT: 1.7 - 2.0



NL: 9.51E4  
TIC F: + c ESI SRM  
ms2 204.215  
[57.428-57.430,  
128.174-128.176] MS  
Quantiva\_Pesticides23

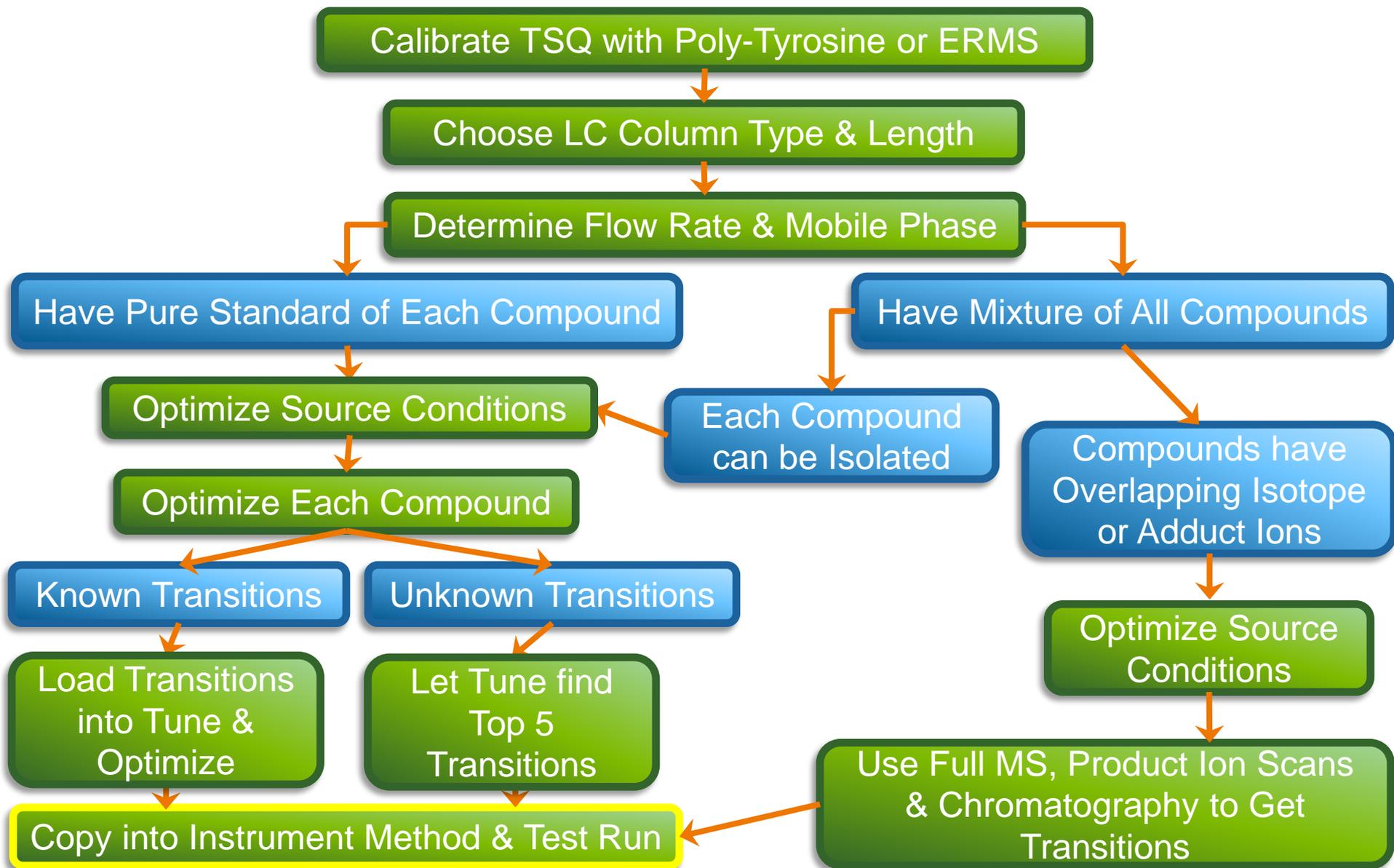
# Q1 Resolution and Chrom Filter

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.

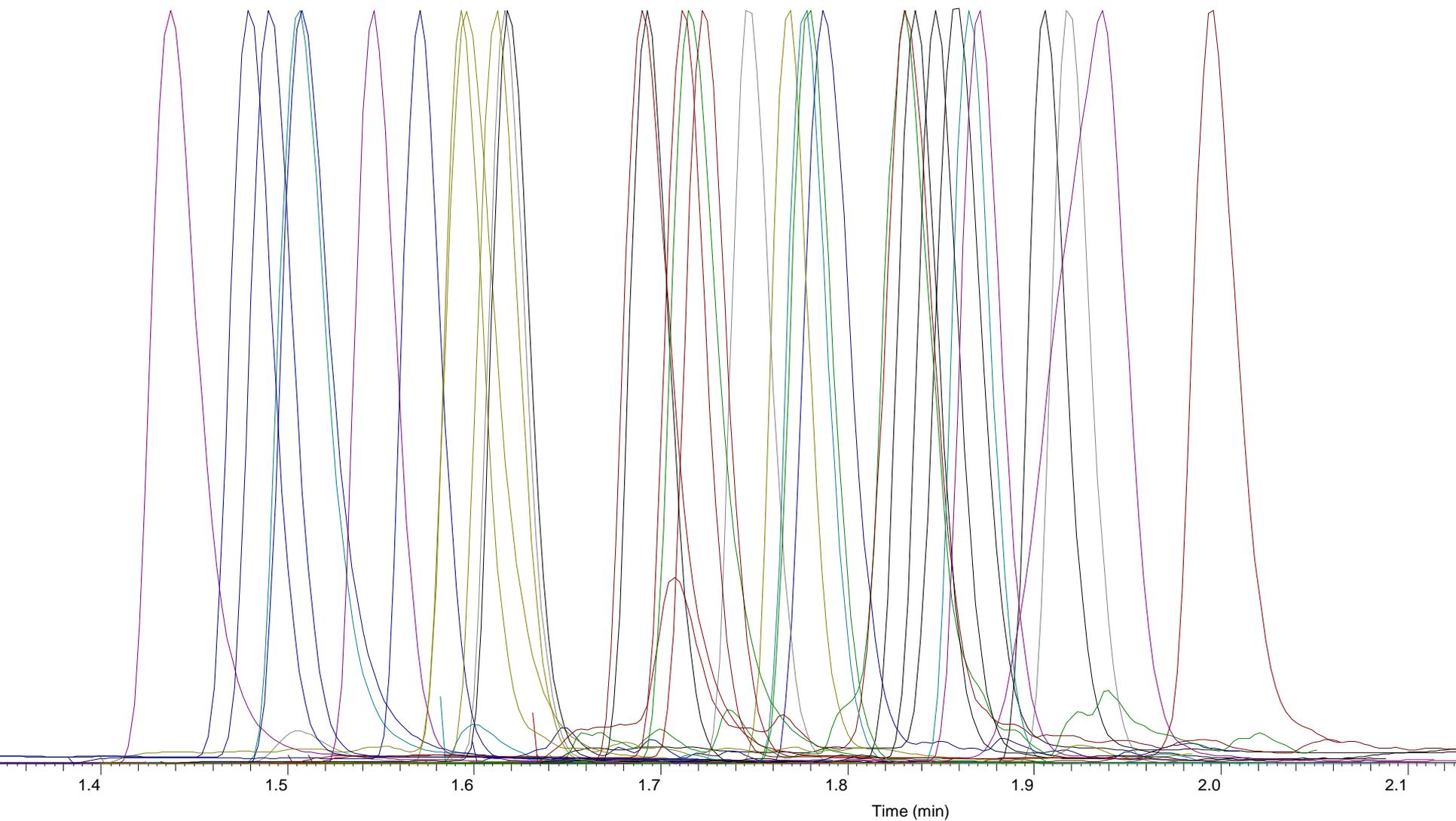


The Chrom Filter is a set of complex algorithms which provide high and low frequency filtering common to all triple quadrupoles. You need to enter the LC full width at half height in seconds.

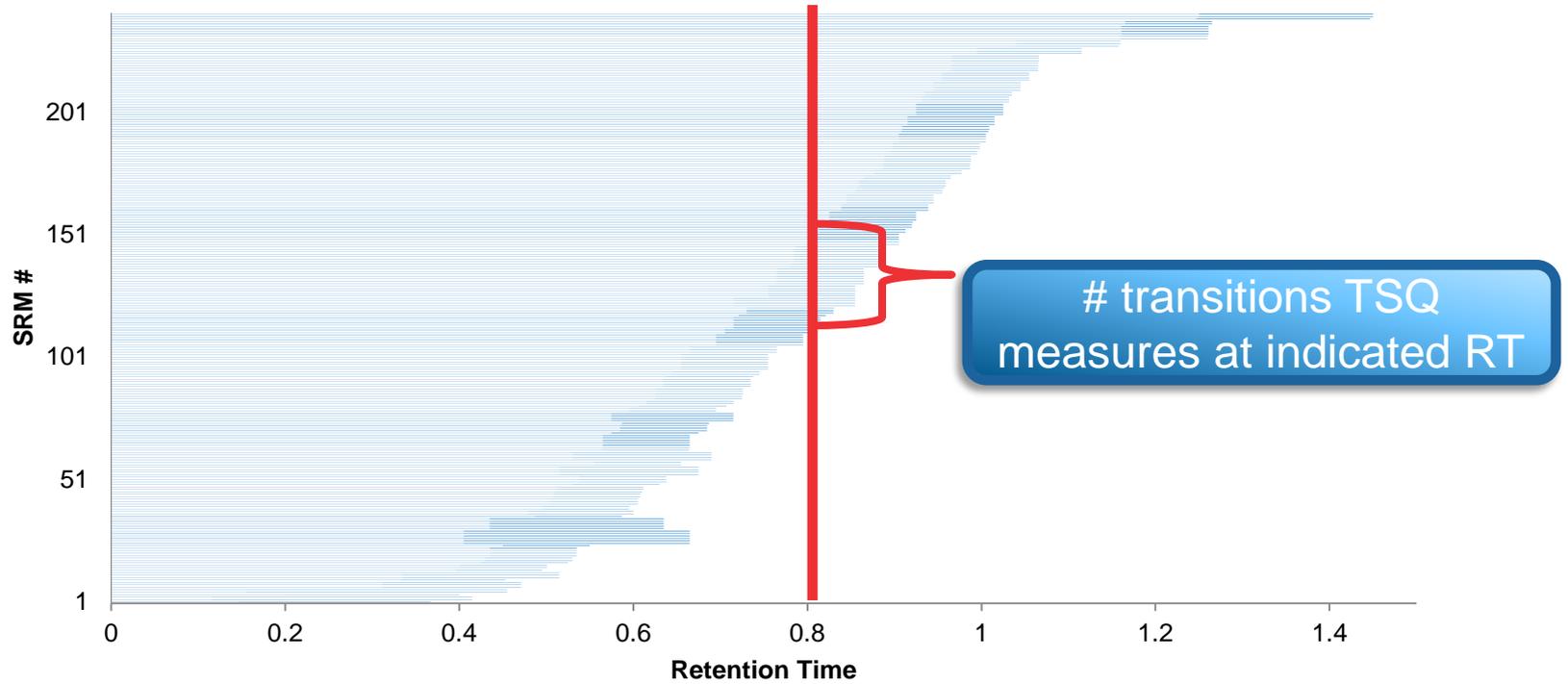
# Test the final LC-MS/MS Method



# Chromatogram of All Components Successfully Quantified



## Timed SRM Chart



# 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

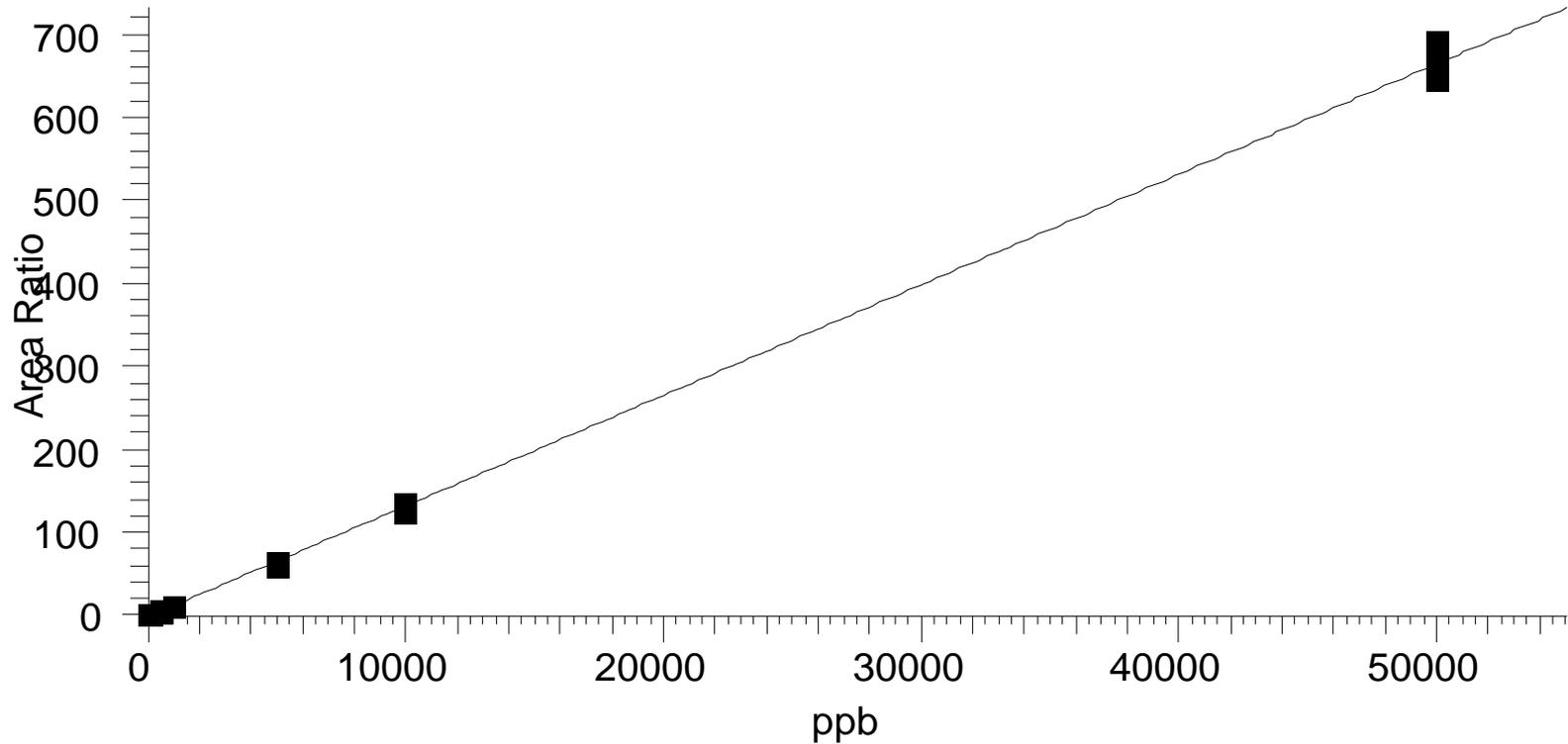
Average Scan by Inst: Yes

Micro Scan Count: 1

# Calibration Curve

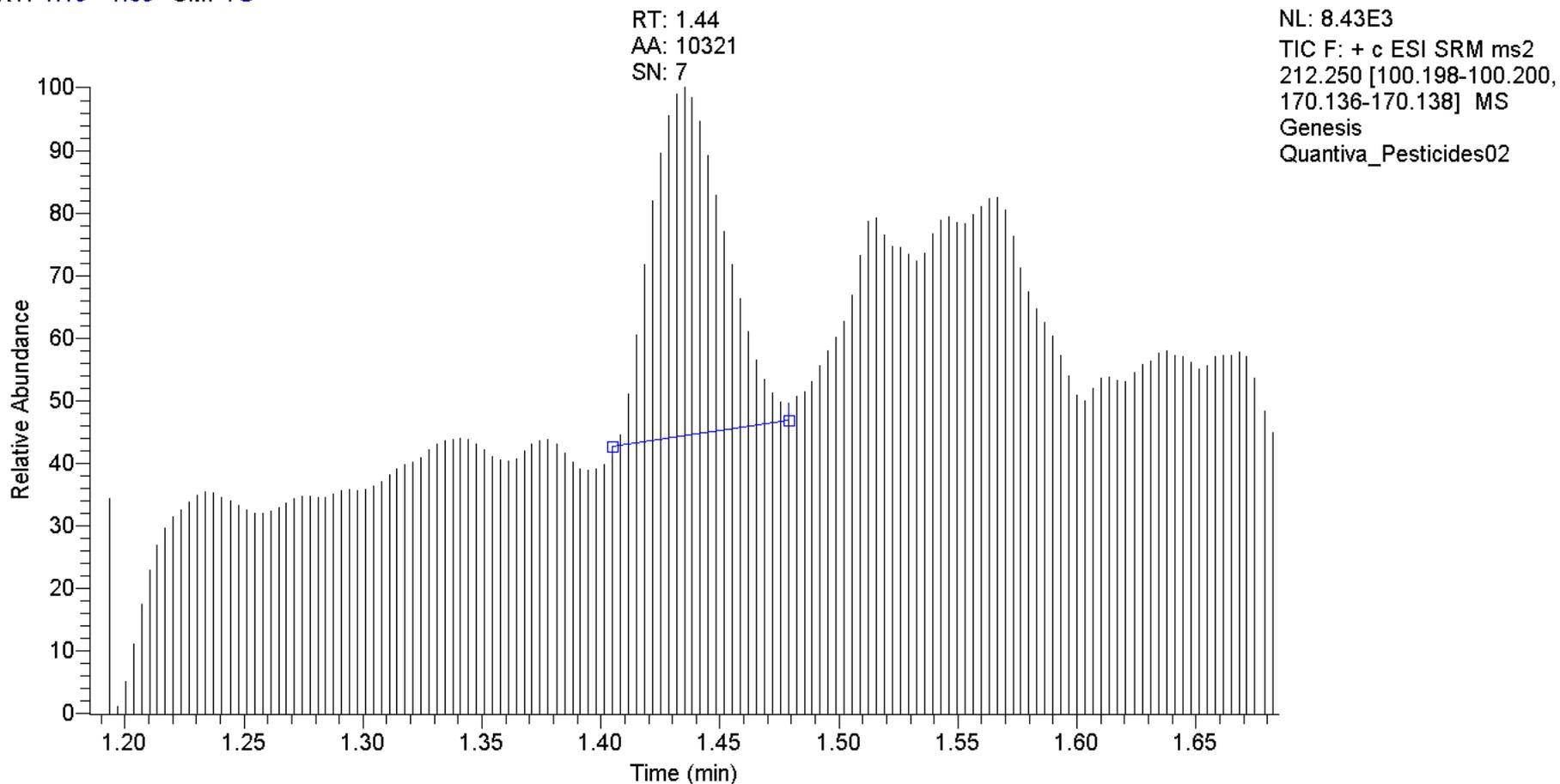
Atraton

$$Y = -1.39356 + 0.0133284 * X \quad R^2 = 0.9994 \quad W: \text{Equal}$$



# LOQ of Atraton (14.7% RSD)

RT: 1.19 - 1.69 SM: 1G



# 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