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# Step by step guide for compound optimization on Xevo TQ-S micro

1. *Manual optimization*
2. *IntelliStart optimization*

# Optimization compounds om TQ

- The optimization of compounds on a TQ MS consists of different stages.
  - Prepare system for infusion
  - Optimize MS 1 with focus on molecular ion (also called parent)
  - Optimize MS 2 with focus on fragmentation and daughter ions
  - Create MS method containing MRM information
  - Shutdown MS

# Manual optimization

# Scope

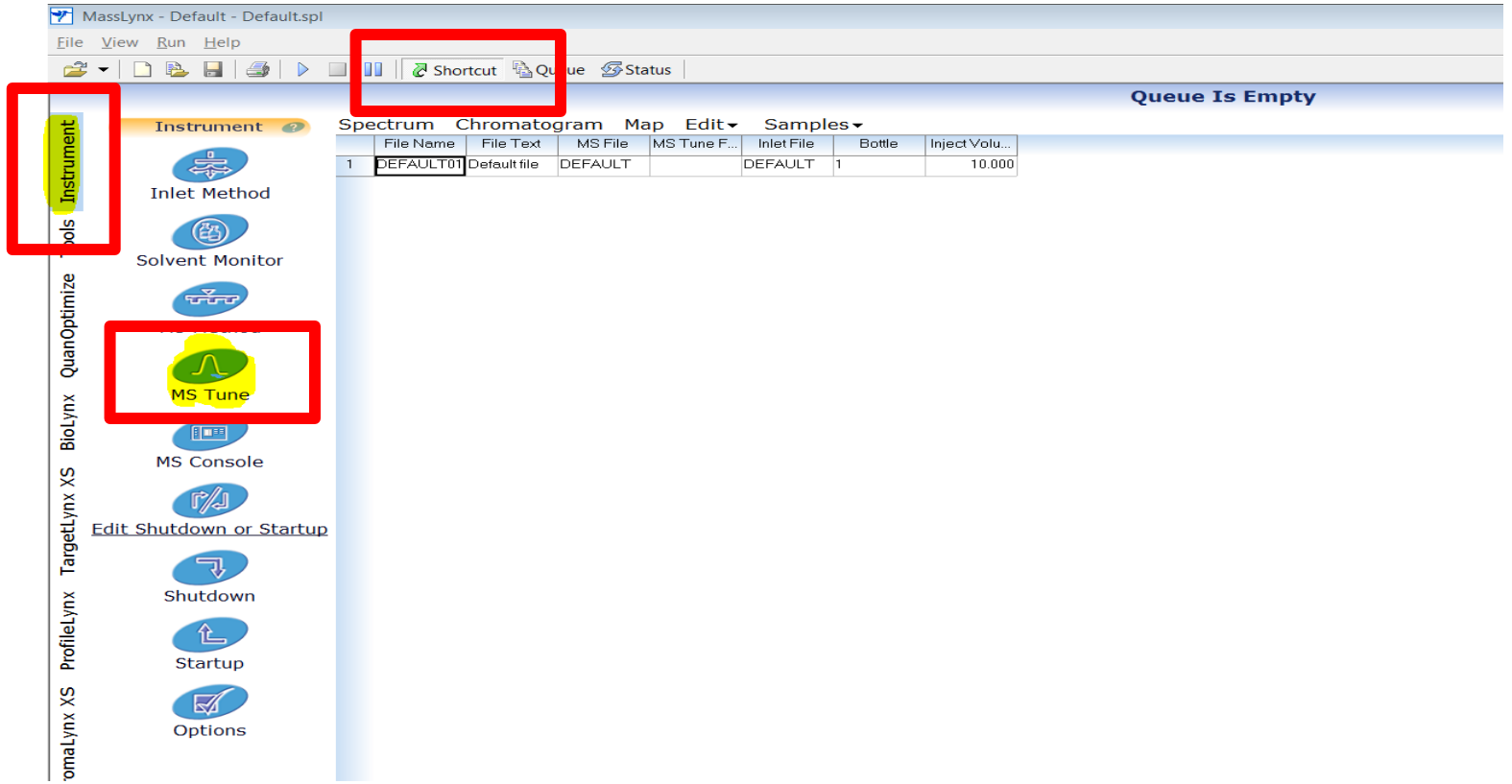
- This presentation serves as a step-by-step guide for manual compound optimization.
- First the steps necessary to **prepare the instrument** are given.
- Then all steps to perform a manual **compound optimization** are explained in detail.
- Finally, the steps necessary to **shutdown the instrument** are highlighted.

# 1. Prepare the instrument

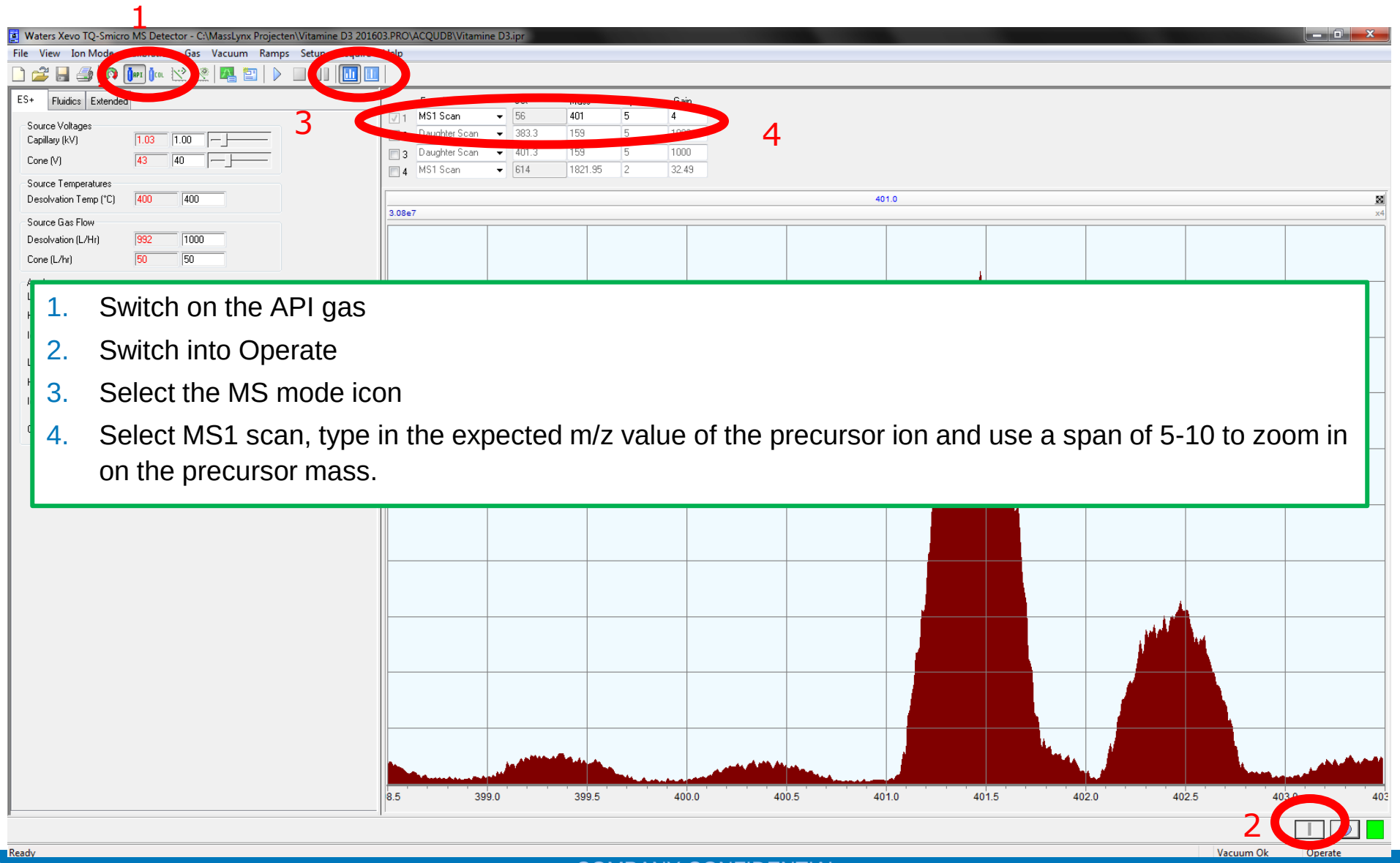
- Access the tune page
- Switch on the instrument
- Prepare the fluidics

# Access the MS Tune

- In the MassLynx main window, click “Instrument” in the left margin and then click the icon “MS Tune”. Make sure the top toolbar icon is “Shortcut”.

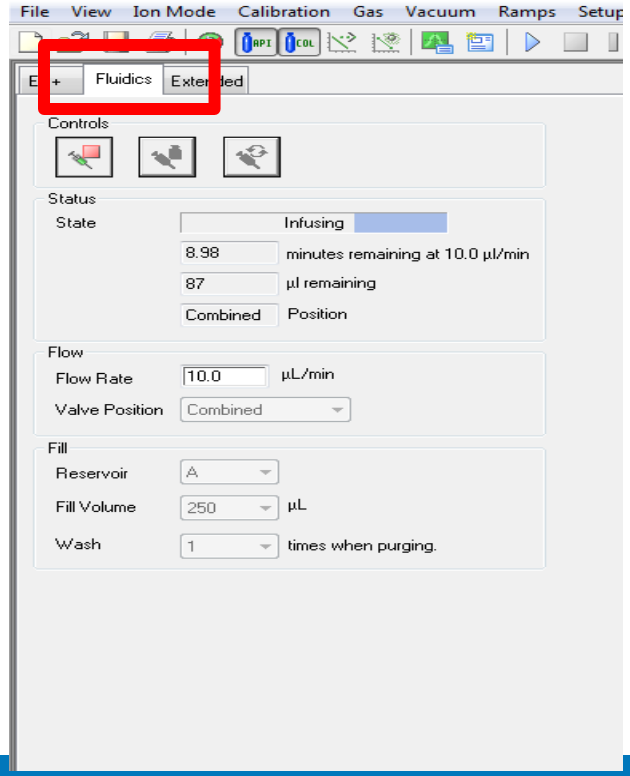


# Step 1: MS1 tune



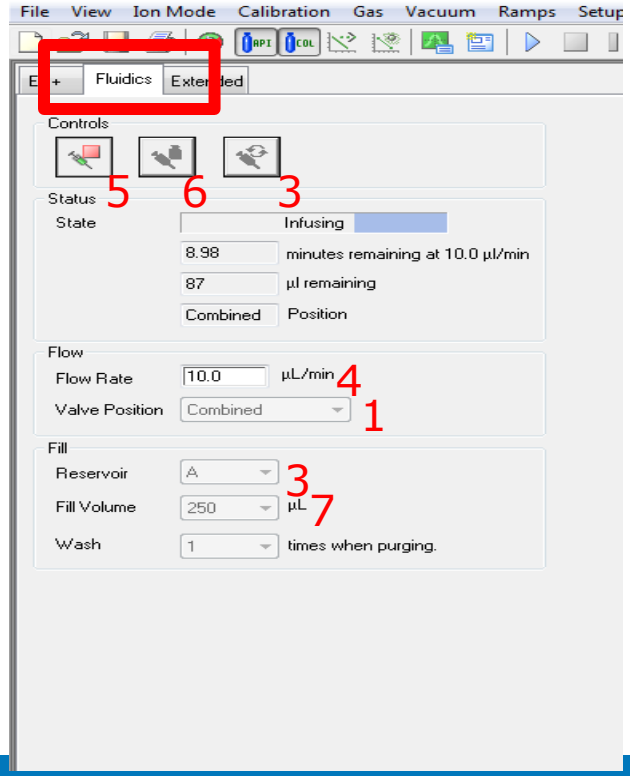
# Fluidics Tab: preparation

- In the **MS Tune** page go to the **Fluidics** tab.
- Make sure the syringe is free from air bubbles. Therefore purge 2-3x with **Wash** solvent prior to first infusion. This will garantuee a stable signal in the spectrum window.
- Prepare a solution of 100-1000 ng/mL in an appropriate solvent (e.g. methanol, acetonitrile). Most convenient is to prepare this in a vial with preslit cap, which can be hung on one of the positions.
- **HINT**: start with 100 ng/mL, use higher concentration in case of low intensity.



# Fluidics Tab: operation

1. Select **Combined** mode
2. Set a mobile phase flow rate of 400 µL/min (access from either the MS Console or Inlet Method editor)
   - Set 80A/20B for early eluters
   - Set 20A/80B for late eluters
   - Set 50A/50B if retention time is not known yet
3. Purge compound prior to infusion from either **reservoir A or B**
4. Set a **Flow Rate** of **10-20 µL/min**
5. Press the **Start** button, the m/z peak of interest should be appearing
6. When the syringe is empty, refill it using the **Refill** button
7. If you have limited volume of sample, use less **Fill Volume**



## 2. Manual compound optimization

- Ionization
- MS1 tune
- MS2 tune

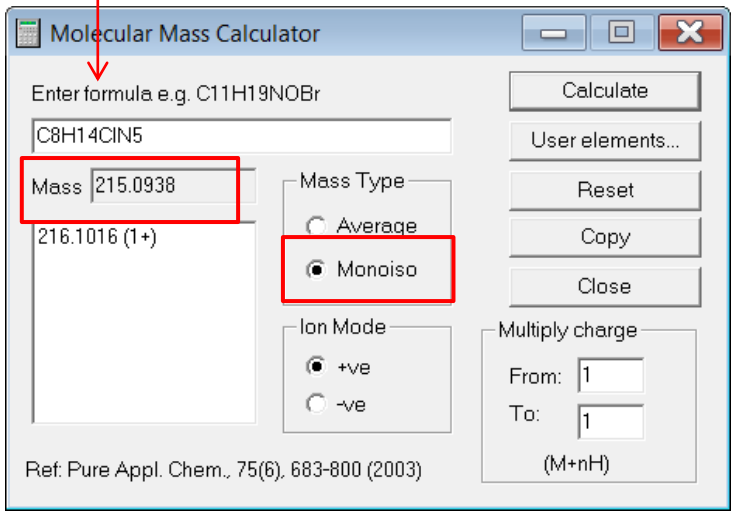
# Step 1: MS1 tune

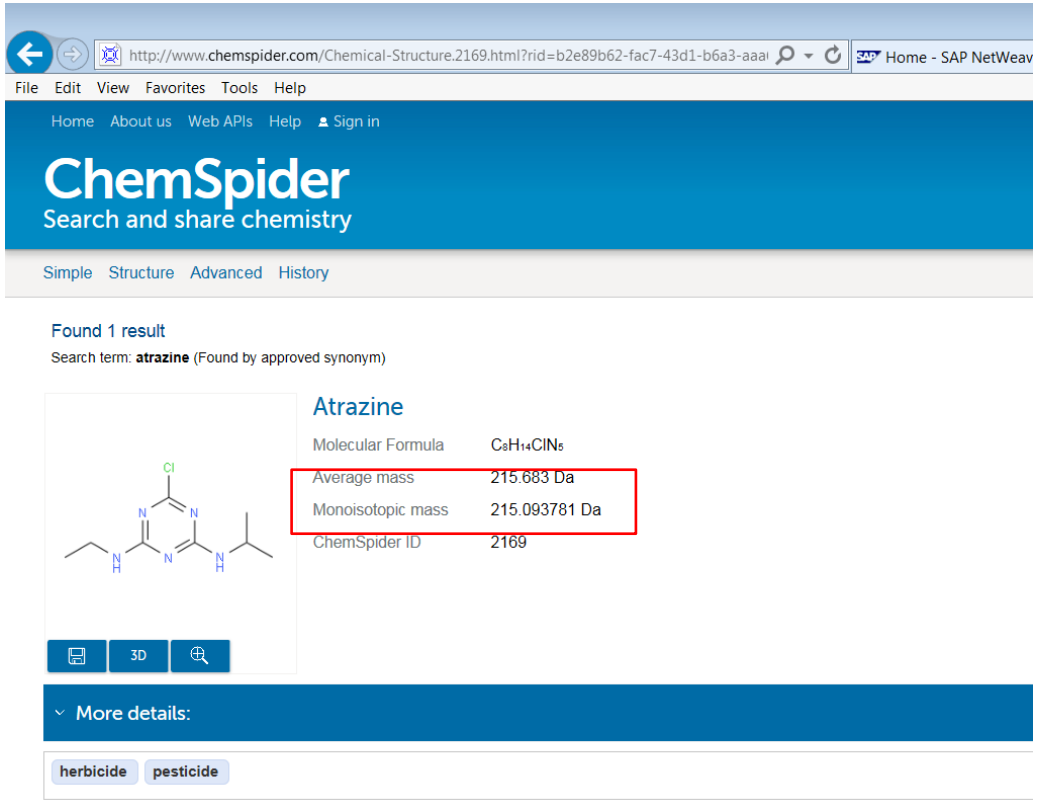
- The goal of this step is to optimize the parent ion (or precursor ion) m/z and corresponding cone voltage, as well as the other source parameters.
- Use Chemspider.com, other internet tools or literature to determine the compound monoisotopic mass [M] (see next slide).
- Source parameters such as desolvation temperature and gas flow can be tuned but will be a compromise for the particular analysis.
- The default ion mode in the tune page is ES+. For other ion modes, go to the toolbar, select *Ion Mode* and make a choice.

# Chemspider and MassLynx MM Calculator

- Chemspider provides a formula, average mass and monoisotopic mass for any given name (see right).

- As an alternative a formula can be entered into the MassLynx Molecular Mass Calculator (see below). Access this windows via tools icon in the left margin of the MassLynx main window.

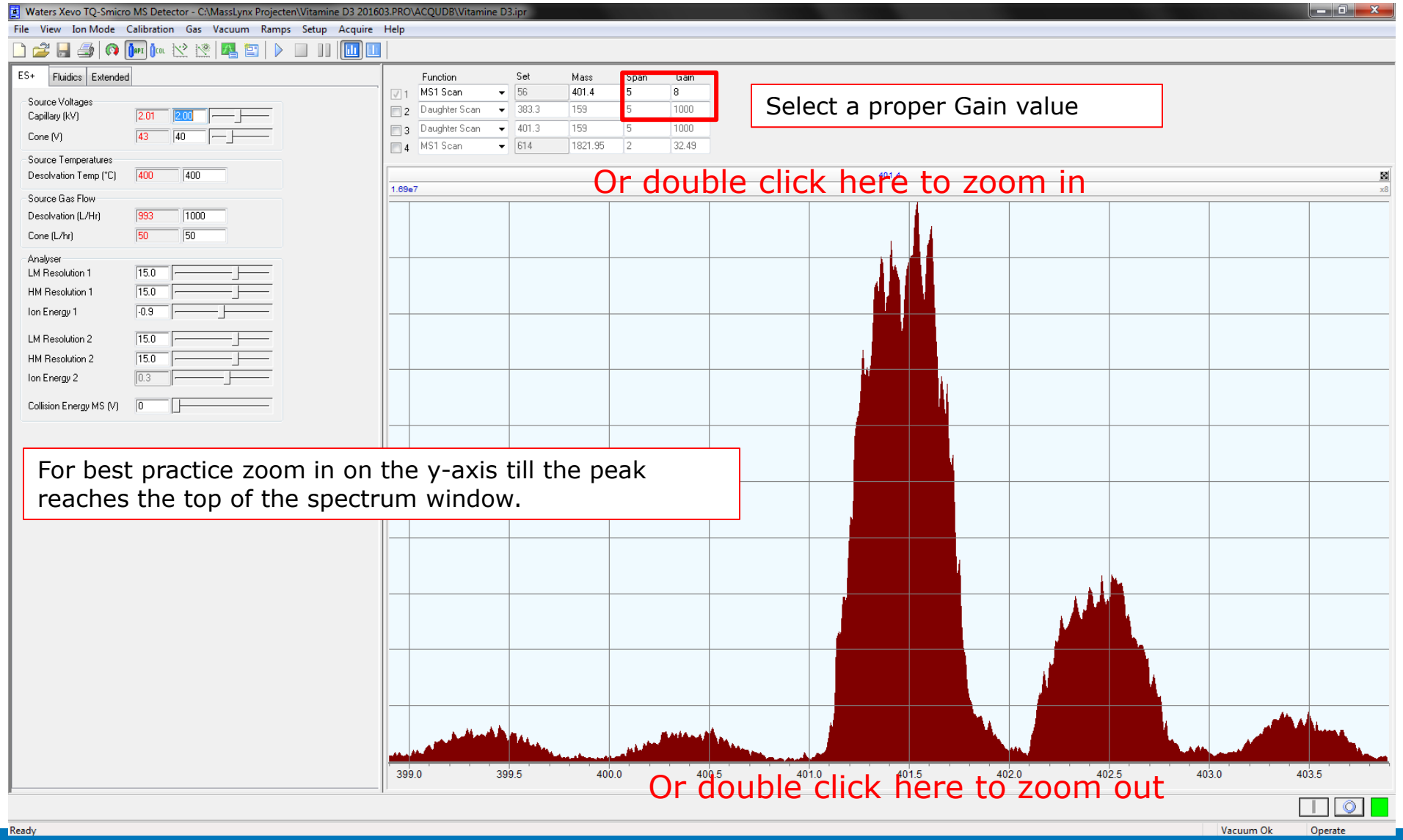


# Parent Ions to be investigated

- In general ES+ provides $[M+H]^+$ = M+1 and ES- results into $[M-H]^-$ = M-1.
- Be aware of the following exceptions, so check for alternative m/z values:
  - Certain ES+ compounds prefer adduct formation:
    - $[M+NH_4]^+$ = M+18
    - $[M+Na]^+$ = M+23
    - $[M+K]^+$ = M+39
  - Certain ES- compounds prefer adduct formation:
    - $[M+Cl]^-$ = M+35
    - $[M+acetate]^-$ or $[M+formate]^-$ = M+45 or M+59
    - $[M+sodium\ formate-H]^-$ or $[M+sodium\ acetate-H]^-$ = M+67 or M+81
  - Certain ES+ compounds fragment easily in the source with loss of:
    - $H_2O$ = M-18
    - MeOH = M-32
    - EtOH = M-46

# Step 1: MS 1 tune



# Step 1: MS 1 tune

- Now the following can be tuned:
  - Capillary voltage (method specific, compromise between compounds)
  - Cone voltage (compound specific)
  - Desolvation temperature (method specific, compromise between compounds)
  - Desolvation gas flow (method specific, compromise between compounds)
  - Soft ionization/transmission mode (compound specific)

# Source Voltages

- **Capillary Voltage: voltage needed to send ions to the surface of the charged droplets, needed for efficient transfer of ions into the gas phase**
  - Compound dependent
  - Optimizes between 0.5 and 3 kV
    - Rarely optimizes above 3kV
    - Values above 3kV in ES- may result into a blue discharge on the capillary tip and should be avoided
  - Needs a compromise in case of multi-compound applications
  - **HINT**: After tuning, make 3 injections at different voltages (0.5kV, 1.5kV, 2.5kV) and compare the S/N. Peak area is not the deciding factor here, because the noise level can increase at lower capillary voltage.

# Source Voltages

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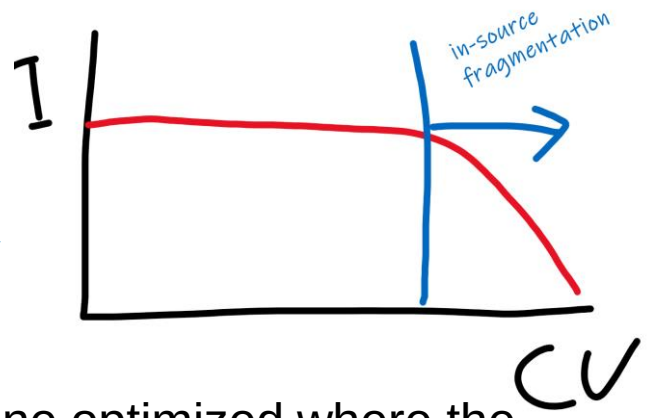
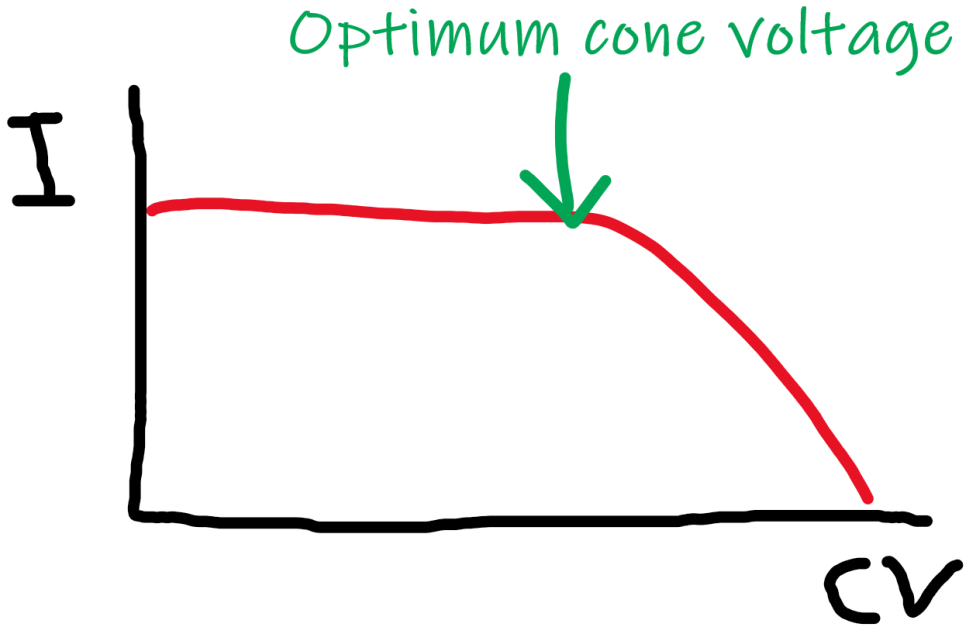
- **Cone voltage: voltage needed to send ions into the first vacuum region**
  - Compound dependent
  - In-source fragmentation occurs at higher values
  - **HINT**: A typical optimization diagram is shown in the figure below. Have the cone optimized where the green arrow is located. At lower cone voltages the peak intensity will be similar, but the noise level will be higher resulting in a lower S/N.



# Desolvation Temperature

- **Desolvation Temperature: temperature of the $N_2$ Flow needed to evaporate mobile phase**
  - Compound dependent
  - Optimizes between 400 °C and 650 °C
  - Depends on mobile flow rate:
    - Regular UPLC flow rates of 400-600 µL/min require desolvation temperatures of around 500°C – 650°C
    - Lower mobile phase flow rates require lower desolvation temperatures
  - Needs a compromise in case of multi compound applications
  - **HINT**: This can be quickly optimized by changing the desolvation temperature in the tune page during infusion. The actual temperature will adapt quickly to the new setting so a live feedback will be given regarding the impact. Just monitor the peak intensity while the temperature changes. Typically, better sensitivities are obtained at higher temperatures.
  - **IMPORTANT**: Be aware that some compounds are thermo labile and should be analyzed at low desolvation temperatures. This should be tested on a case-by-case study.

# Source Gas Flow

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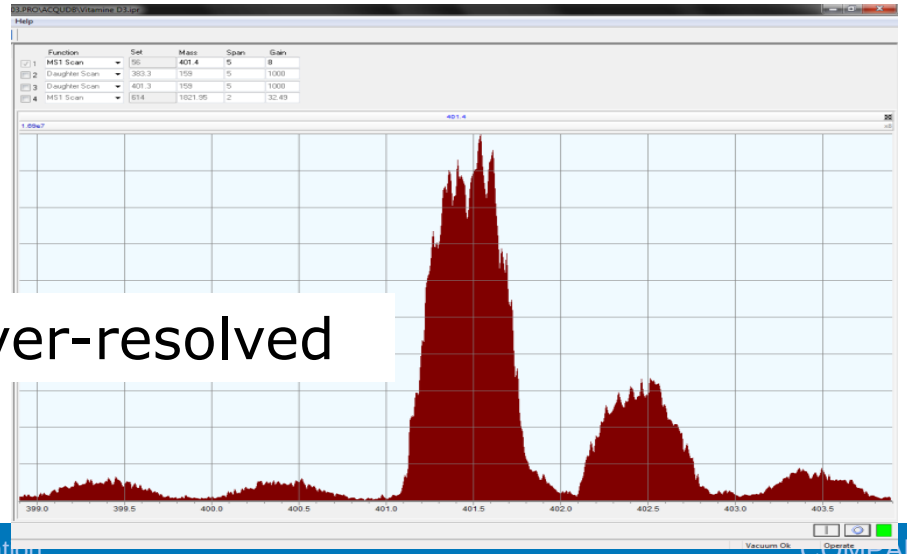
- **Desolvation Gas Flow: $N_2$ flow at high temperature needed to evaporate mobile phase**
  - Compound dependent
  - Optimizes between 800 L/hr and 1200 L/hr
  - Depends on mobile flow rate
    - Regular UPLC flow rates of 400-600 µL/min require desolvation flow rates of around 1000 L/hr - 1200 L/hr
    - Lower mobile phase flow rates typically require lower desolvation flow rates
  - Needs a compromise in case of multi compound applications
  - **HINT**: Generally, a setting of 1000 L/h is already optimal. There is not much to be gained with this setting.

- **Cone Gas Flow: counter-flow of $N_2$ to protect cone from getting dirty and to break down possible unwanted clusters or adducts**
  - Use a value of 50 L/hr or higher for protection of cone
  - **HINT**: When working with ESI the default setting of 50 L/hr can be used. When using Unispray this is best optimized by performing injections at different gas flow rates (ie 0, 50, 100 L/hr) and check the S/N ratios. When no difference is observed, chose the higher setting.

# Resolution Settings (not to be tuned!)

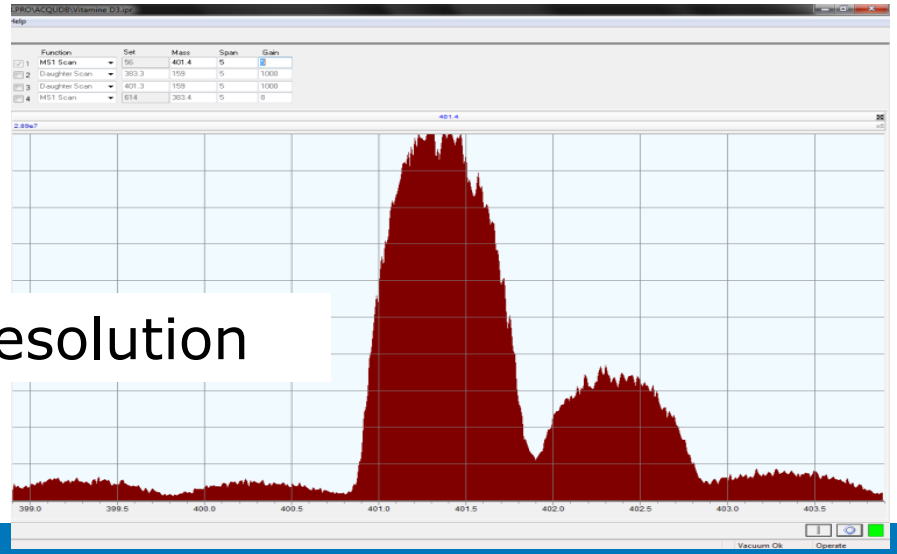
- **LM Resolution 1 and HM Resolution 1**
  - **The resolution settings should be set once a year after PM and must kept the same for all methods on the same MS. The resolution settings should be provided by a Waters engineer after installation or PM.**
    - You should check if the resolution settings are OK. Aim at unit resolution, *i.e.* 1 Da at the base line
    - LM Resolution has a higher impact on low masses, while HM Resolution has higher impact on high masses
  - Examples below:

Left: LM Res 20, HM Res 15



Slightly over-resolved

Right: LM Res 15, HM Res 15



Unit Resolution

# Ion Energies (not to be tuned!)

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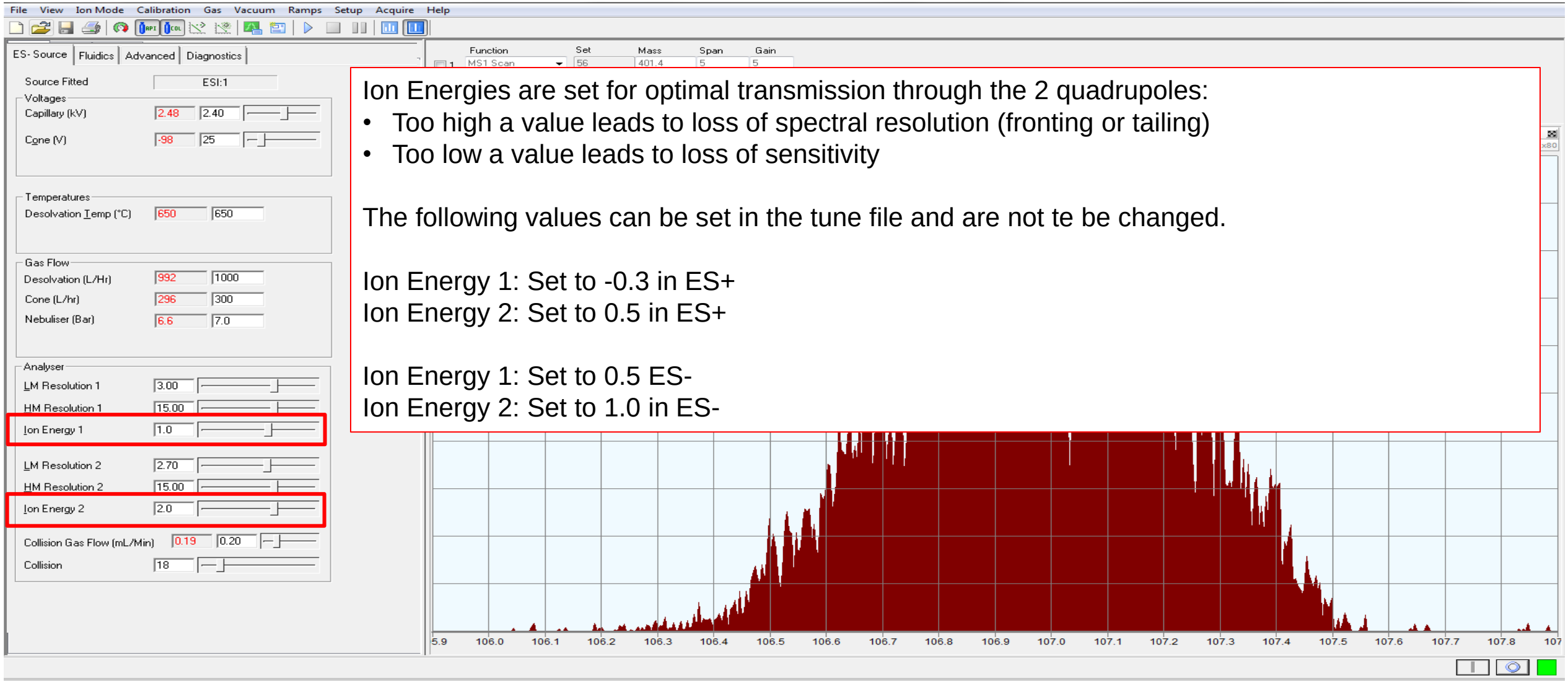



Ion Energies are set for optimal transmission through the 2 quadrupoles:

- Too high a value leads to loss of spectral resolution (fronting or tailing)
- Too low a value leads to loss of sensitivity

The following values can be set in the tune file and are not te be changed.

Ion Energy 1: Set to -0.3 in ES+
Ion Energy 2: Set to 0.5 in ES+

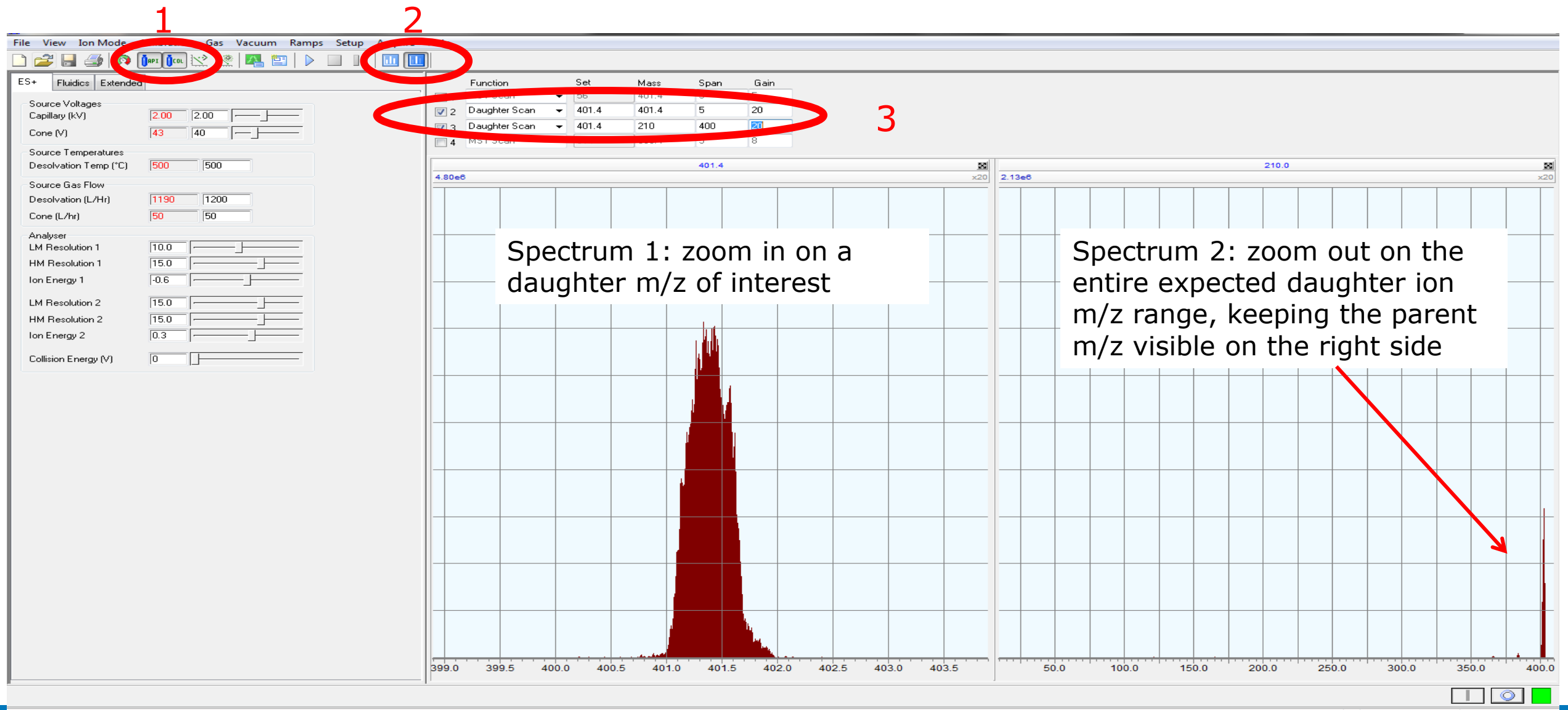
Ion Energy 1: Set to 0.5 ES-
Ion Energy 2: Set to 1.0 in ES-

# Step 2: MS2 tune

1. Switch on the collision gas
2. Select the MSMS mode icon
3. Select 2 times daughter scan, type in 2 times the found precursor m/z value under set. Zoom in on the precursor m/z in one spectrum window and zoom out on the entire range in the other spectrum window

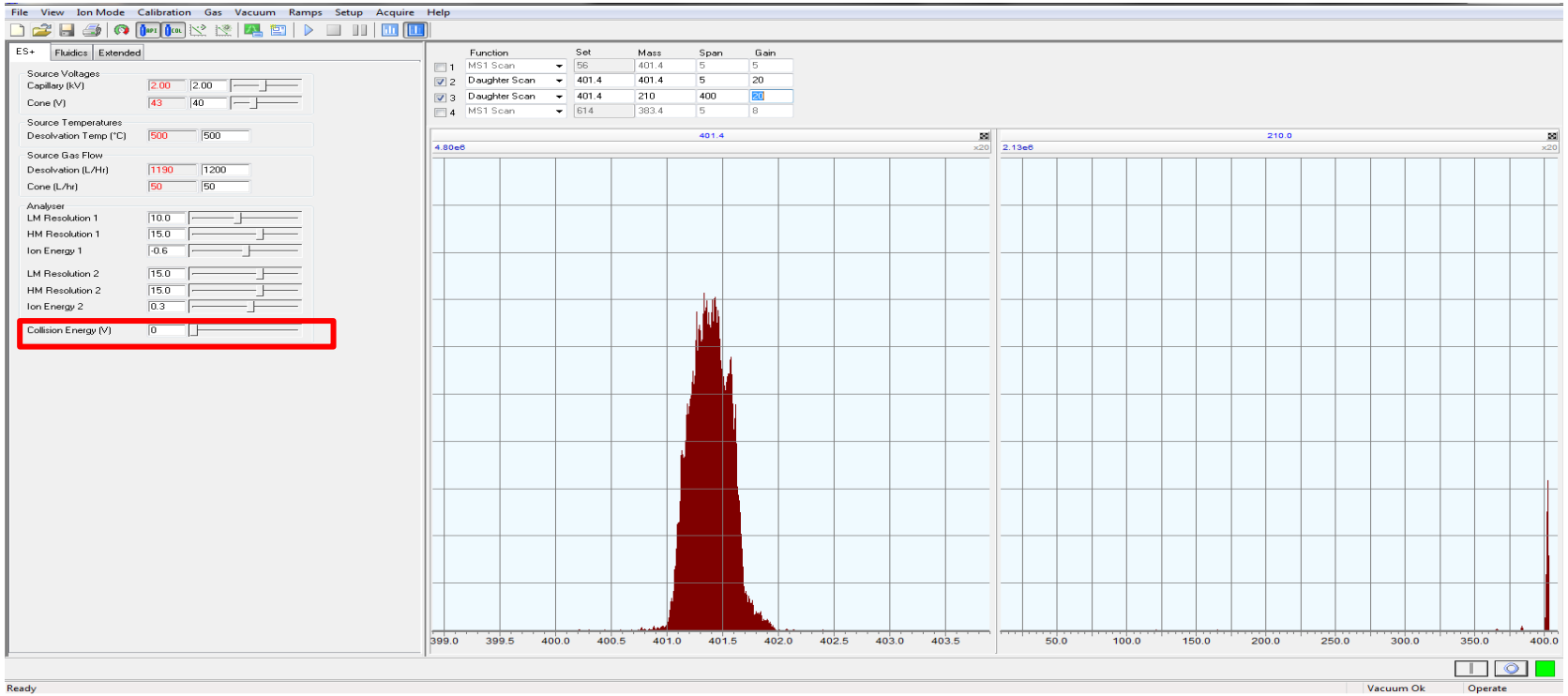
- The goal of the step 2 is to optimize daughter ion (or product ion) m/z and their corresponding collision energies.
- This is done by selecting daughter scan from the dropdown menu above the spectrum window.
- Whenever possible, optimize multiple fragments and select the best ones at the end of the validation stage.
- Fragment ion can be selected by manually changing the collision energy.
- For complex fragmentation patterns, multiple daughter ion spectra data can be acquired manually, and spectra can be compared.
- Daughter ion m/z and collision energies can be changed between different compounds in an MS method.
- All other parameters will be a best compromise for the particular application.

# Step 2: MS2 tune



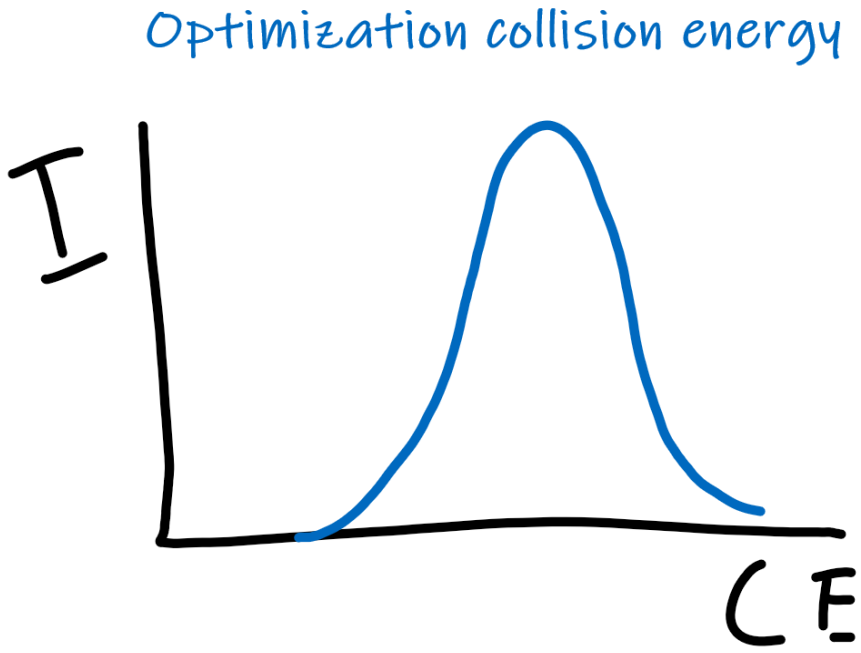
# Step 2: MS2 tune

- **HINT**: Start at a very low collision energy (you will predominantly see the parent m/z) and gently increase the energy up to roughly 60eV and see which daughters of interest occur in the right window. Aim at daughters with highest intensity.
  Using the left window select a daughter m/z of interest and optimize the collision energy when the maximum response is observed. Determine the m/z at 1 decimal.



# Step 2: MS2 tune

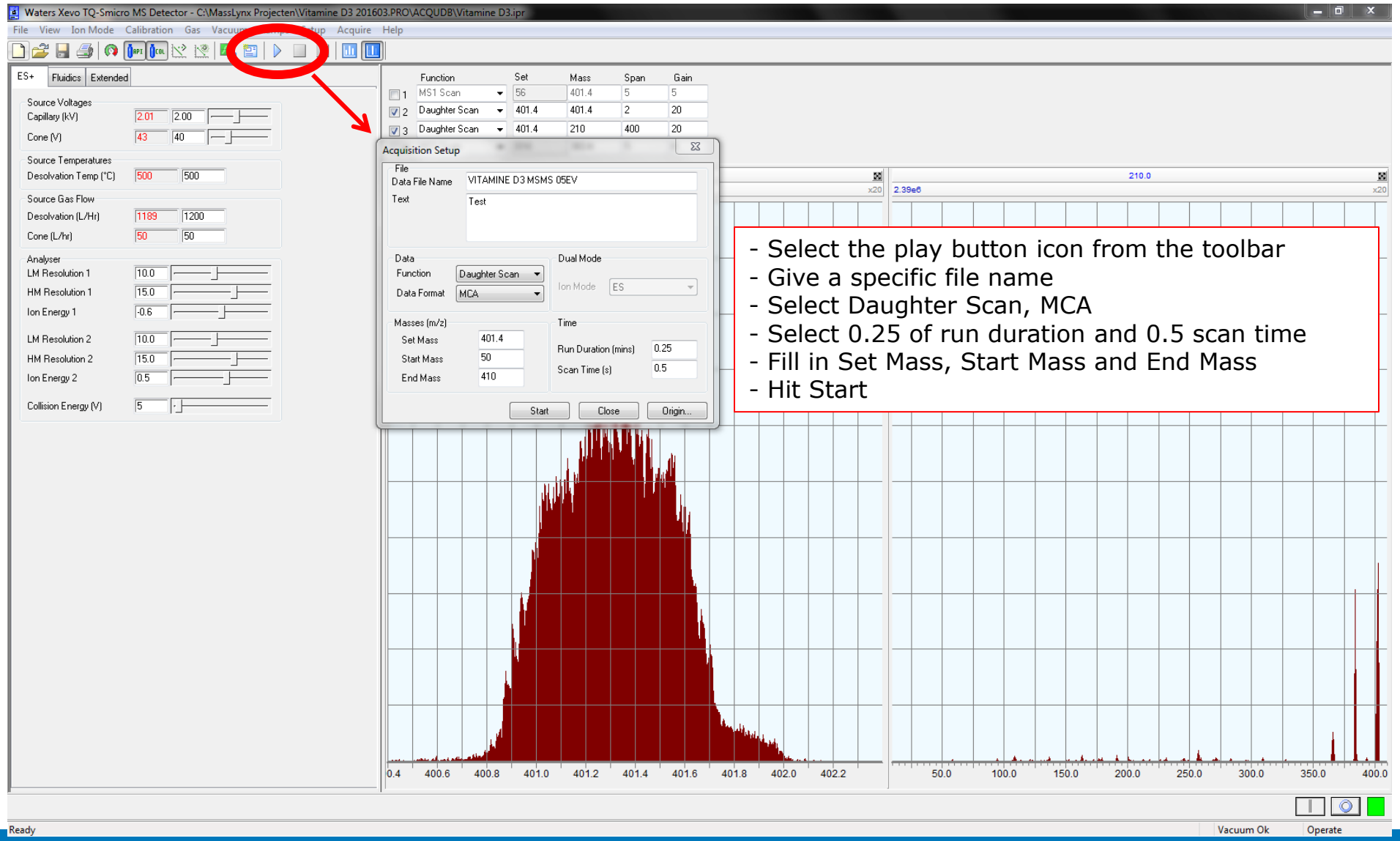
- The collision energy typically optimizes as follows:



# Step 2: MS2 tune: Manual acquisition of daughter ion spectra

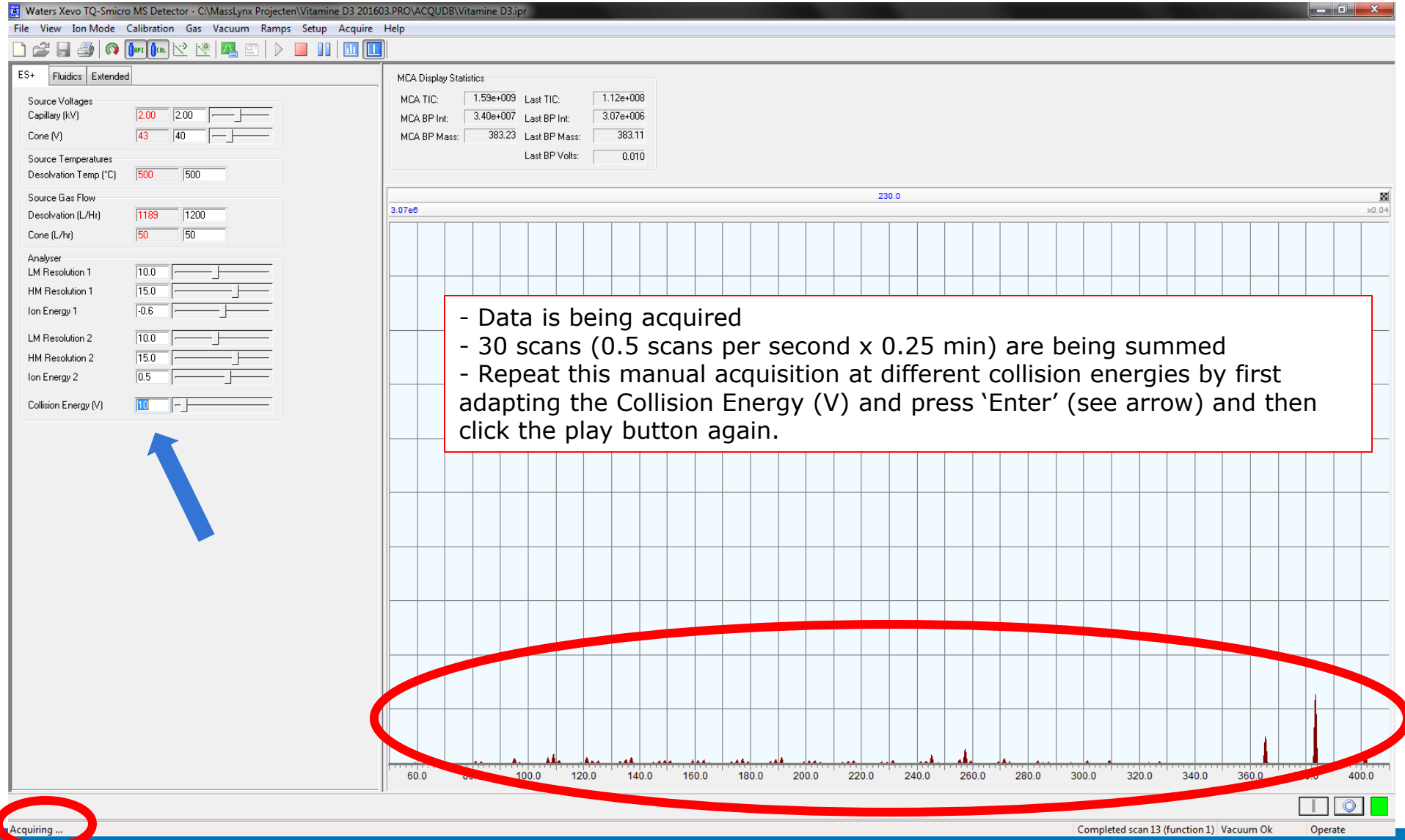
- Acquisition of MS2 spectra and daughter spectra can also be carried out. See the procedure on the following pages.

# Manual acquisition of daughter ion spectra



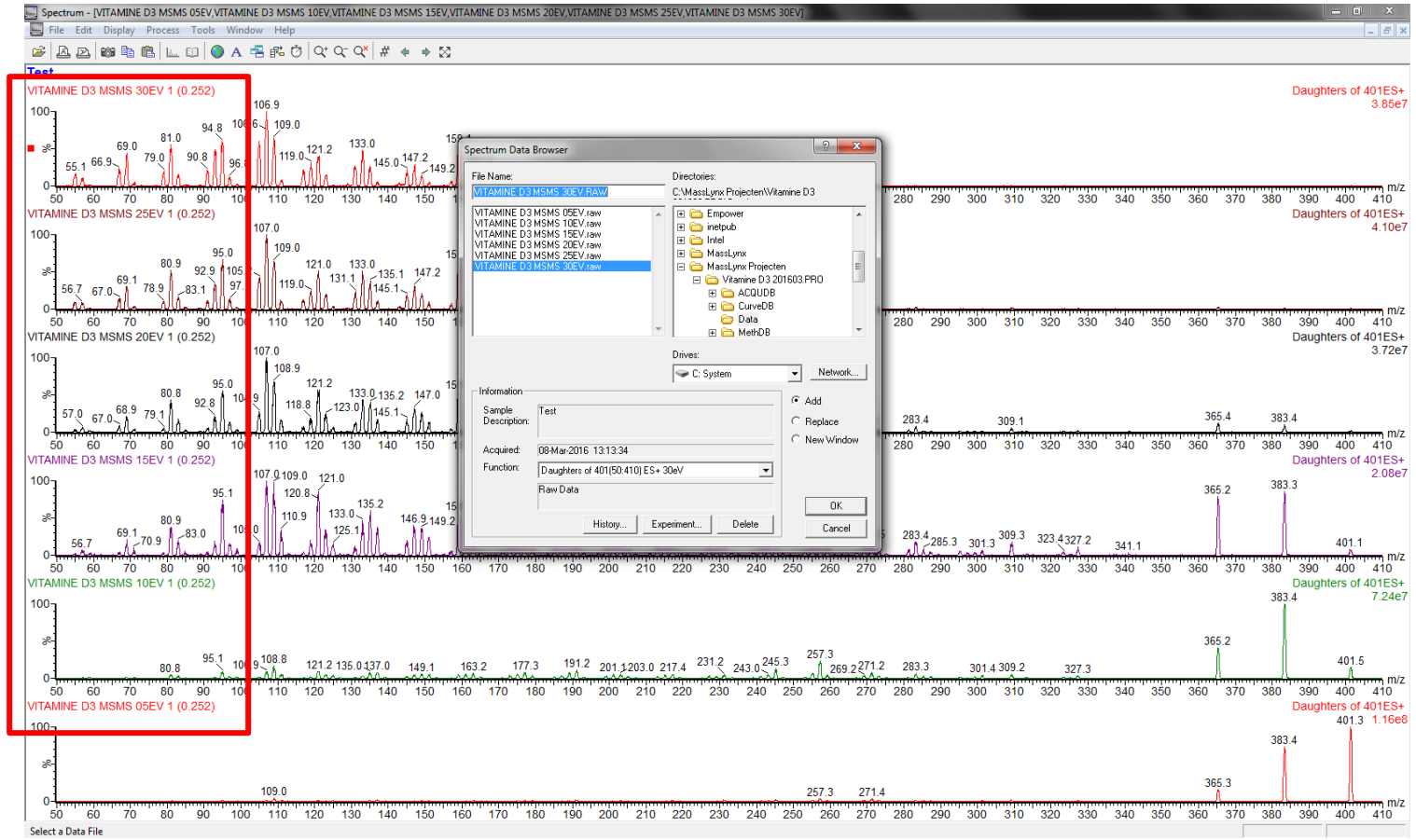

- Select the play button icon from the toolbar
- Give a specific file name
- Select Daughter Scan, MCA
- Select 0.25 of run duration and 0.5 scan time
- Fill in Set Mass, Start Mass and End Mass
- Hit Start

# Manual acquisition of daughter ion spectra



# Manual acquisition of daughter ion spectra

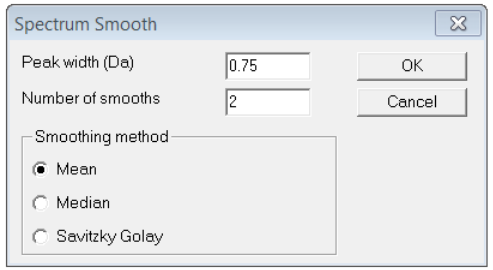
- Go to the sample list and select spectrum from the toolbar
- Open all acquired spectra

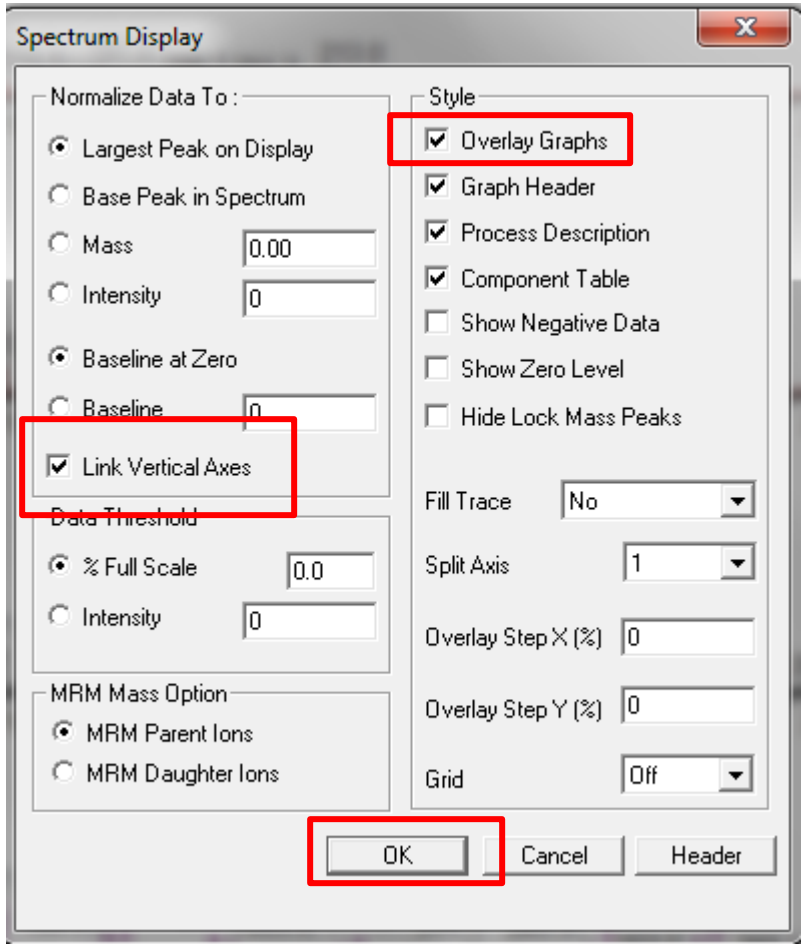


# Manual acquisition of daughter ion spectra

- For best comparison, align and overlay the spectra
- In the spectrum toolbar > display > view
- Select *Link Vertical Axes*
- Select *Overlay Graphs*
- Press *OK*

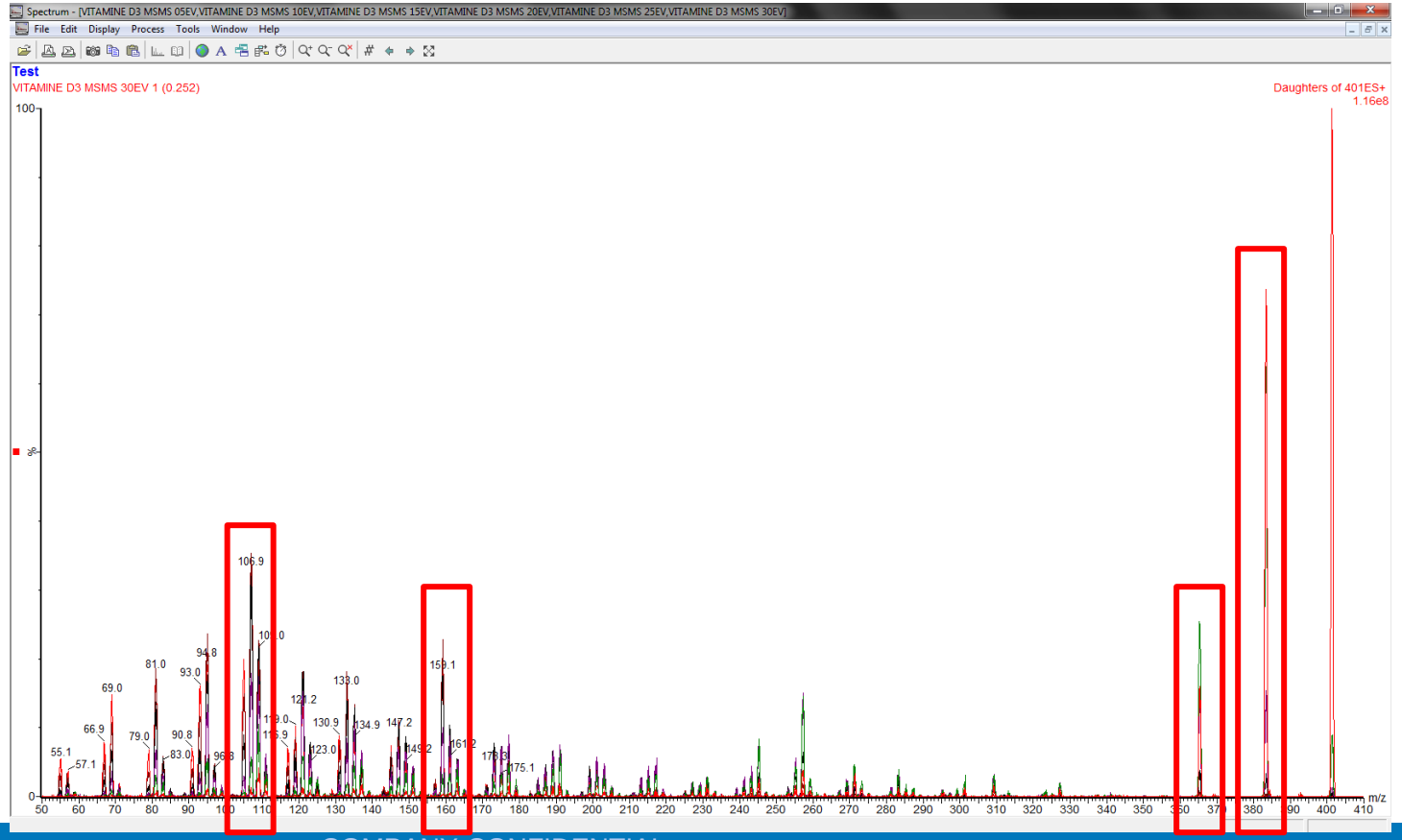
- Prior to this, you can smooth all spectra
- In the spectrum toolbar > process > smooth
- Fill in the following criteria
- Press *OK*



# Manual acquisition of daughter ion spectra

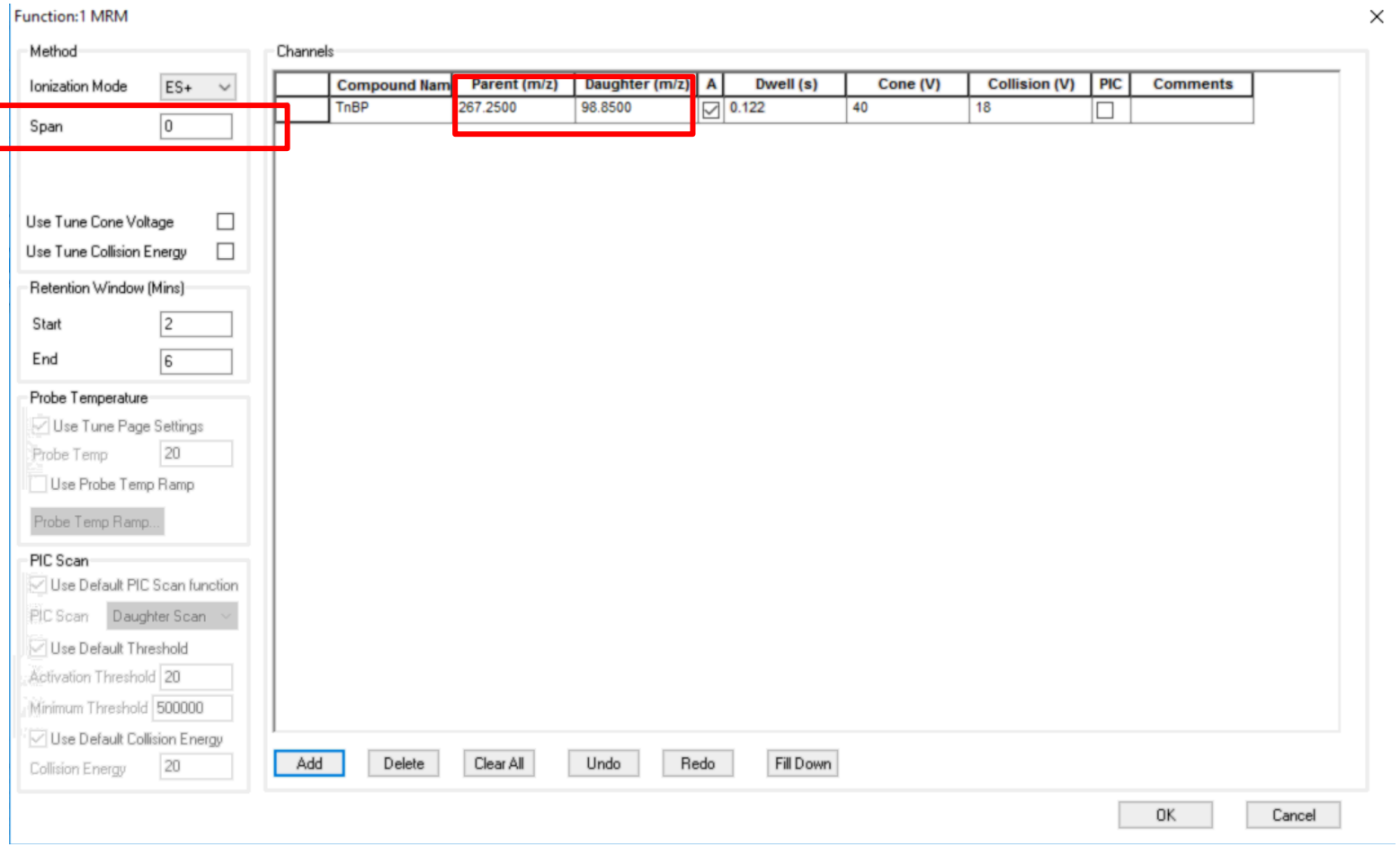
- An overlaid and aligned spectrum is seen
- This allows for best comparison of the fragment ions
- Select up to 4-5 fragment masses for fine tuning
- Therefore go back to the tune page



# Additional remarks regarding the MS Method

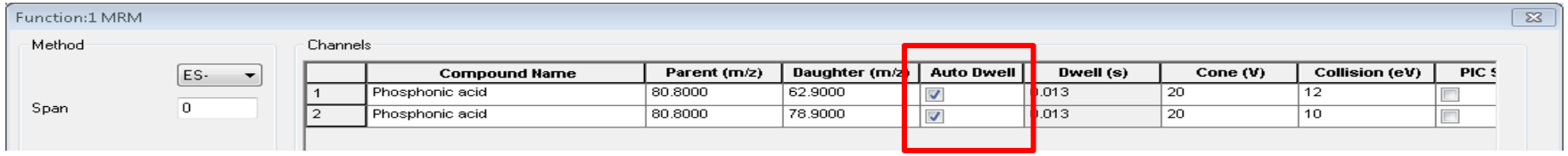
# MS Method

- Fill in Parent m/z and Daughter m/z value with 50mDa accuracy.
- If m/z values are selected correctly from the tune page spectrum window, a span around the m/z value is not needed. It is therefore recommended to set this value to 0.

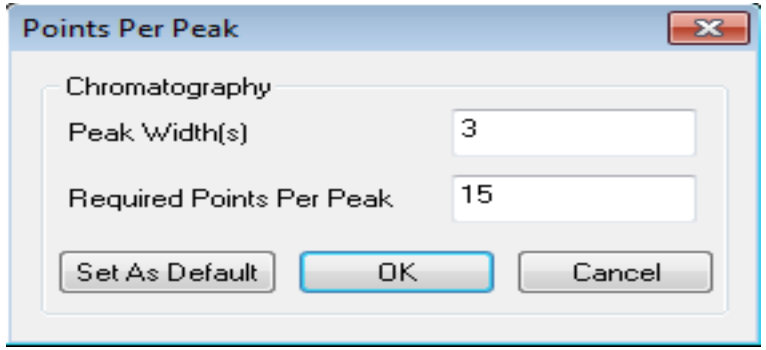


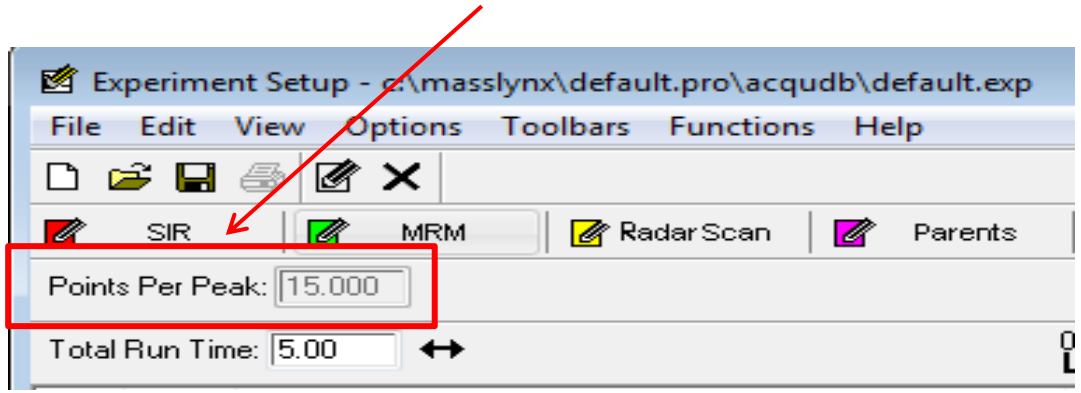
# Auto dwell

- Select auto dwell for an automatic calculation of the dwell time depending on the number of simultaneous transitions (and inter-scan delay times, polarity switching) and the specified points per peak.




- When using the auto dwell option, adjust your peak width and specify +/- 15 points per peak for quantitative analysis (options MS method). The dwell time will be adjusted accordingly. Please verify the actual points per peak of the method.



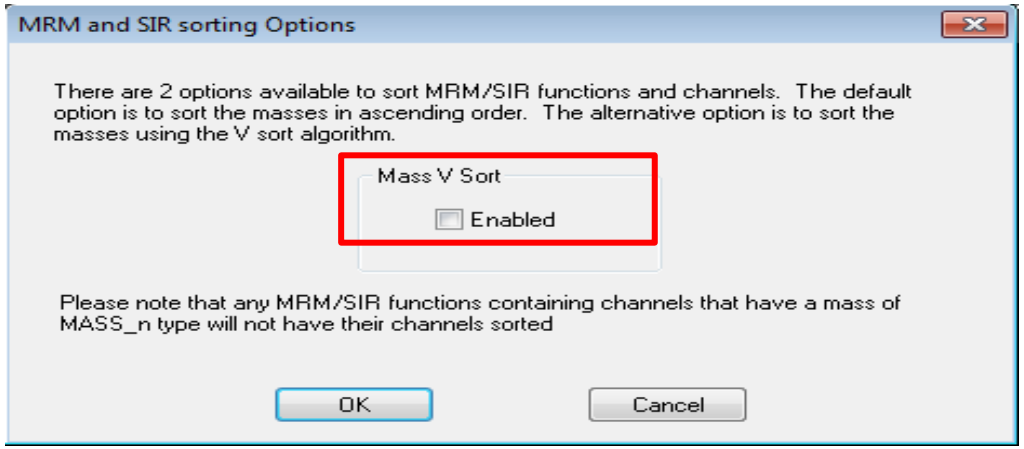



# MRM V-Sort

- There are two options for sorting out the MRM transitions:
    1. acquiring masses in ascending order
    2. V-sort algorithm (acquired in the optimum order of performance, minimizing large voltage jumps)

- This can be important when very short dwell times and inter-scan delay times are used. It is recommended to always use V-sorting as this will never have a negative impact on sensitivity.

- Applying the v-sorting will affect the MS method as it will reorder the functions. It may have an impact on the processing method, though, when the acquisition function numbers are set to 0 there will be no problem.

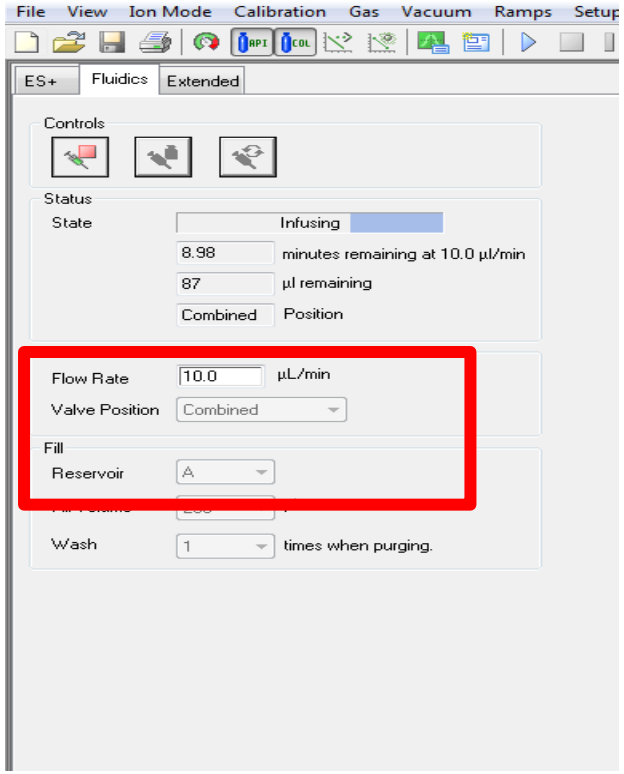


# 3. Shutdown the instrument

- Clean the fluidics
- Switch off the instrument

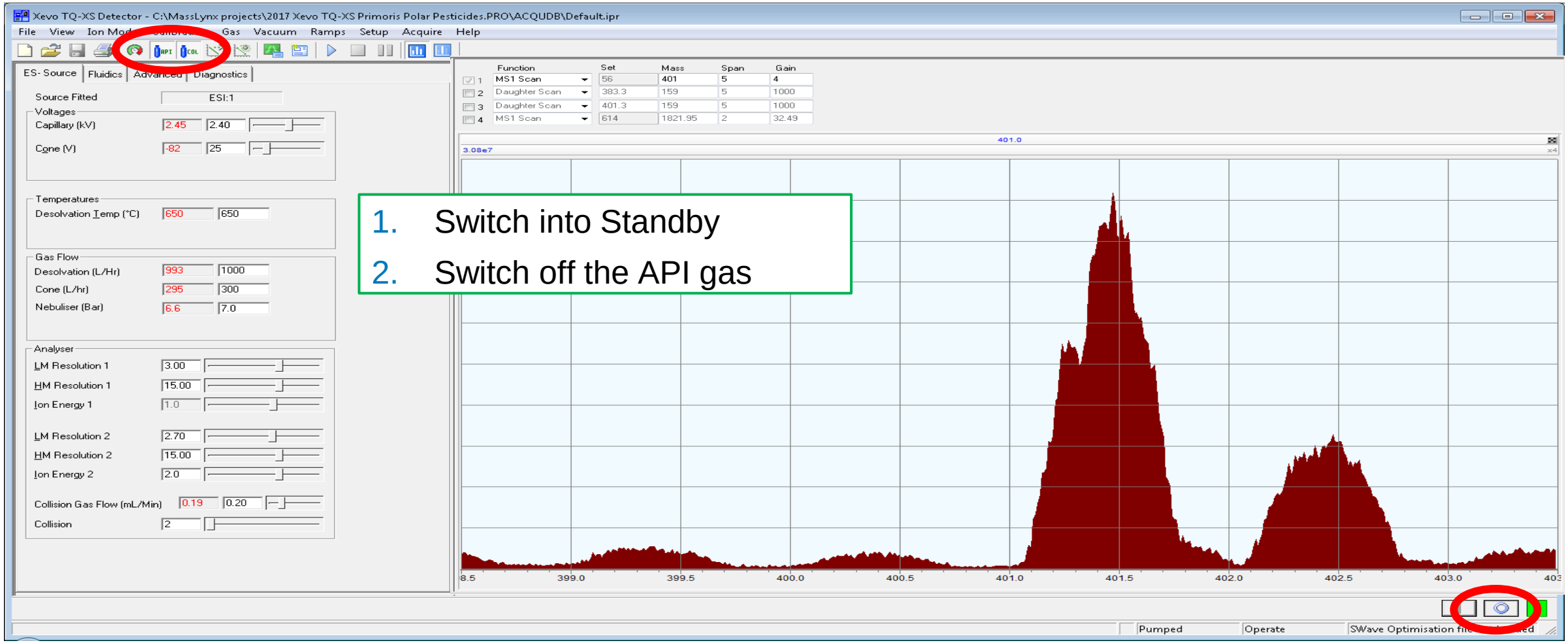
# Fluidics Tab

- In the MS Tune page go back to the Fluidics tab. Replace the active reservoir by a bottle of pure methanol and purge to prevent blockage of the valve and to keep the fluidics system clean.
- After purging, press start in the combined position to flush the valve internal connections & tubing. Keep the flow on for 30s.
- Stop the flow. Leave the bottle of methanol in the actual position.
- Stop the LC mobile phase flow.



# Switch off the instrument

2




1. Switch into Standby
2. Switch off the API gas

1

# Intellistart optimization

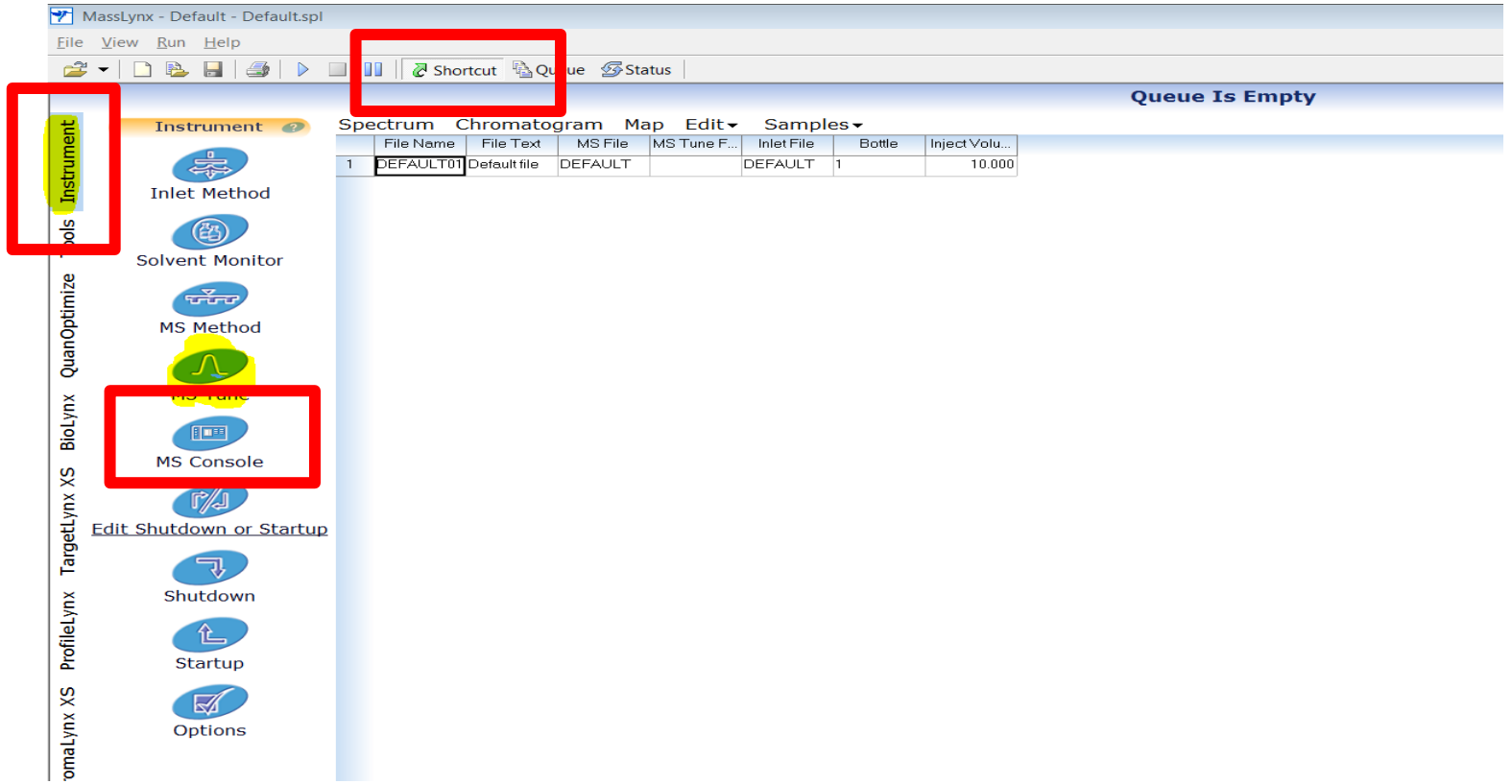
# Scope

- This presentation serves as a step-by-step guide for manual compound optimization.
- First the steps necessary to **prepare the instrument** are given.
- Then all steps to perform an IntelliStart automatic **compound optimization** are explained in detail.
- Finally, the steps necessary to **shutdown the instrument** are highlighted.

1. **Prepare the instrument**
   - **Access the Console**
   - **Switch on the instrument**
   - **Prepare the fluidics**

# Access the Console

- In the MassLynx main window, click “Instrument” in the left margin and then click the icon “MS Console”. Make sure the top toolbar icon is “Shortcut”.



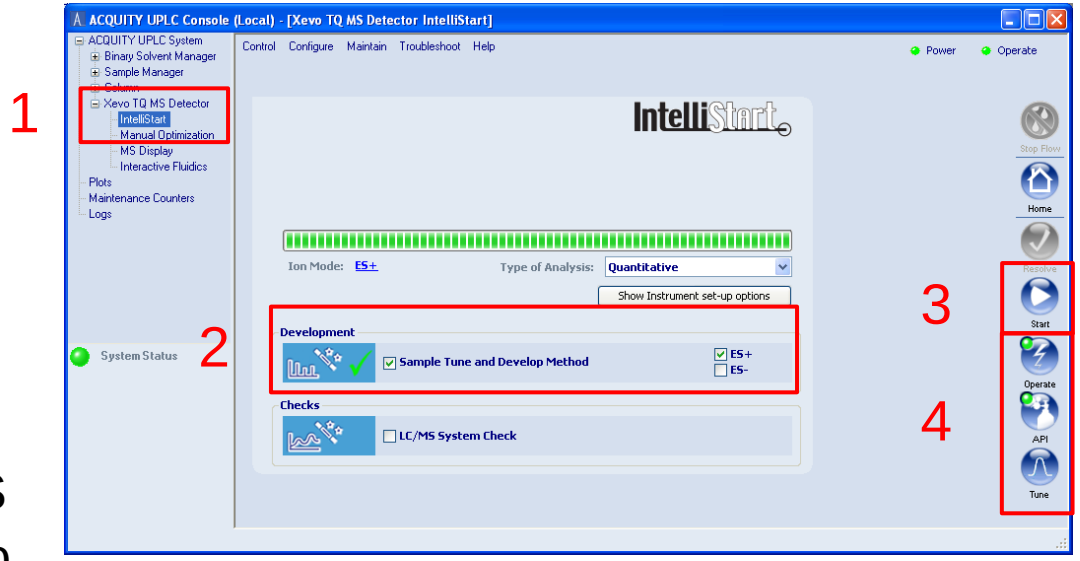
# Console

1. In the Console, access IntelliStart via the toolbar on the left: Xevo TQ-XS MS Detector > IntelliStart.

2. Select “Sample Tune and Develop Method”. Select the polarity of choice: ES+/ES-/both (or US+/US-/both).

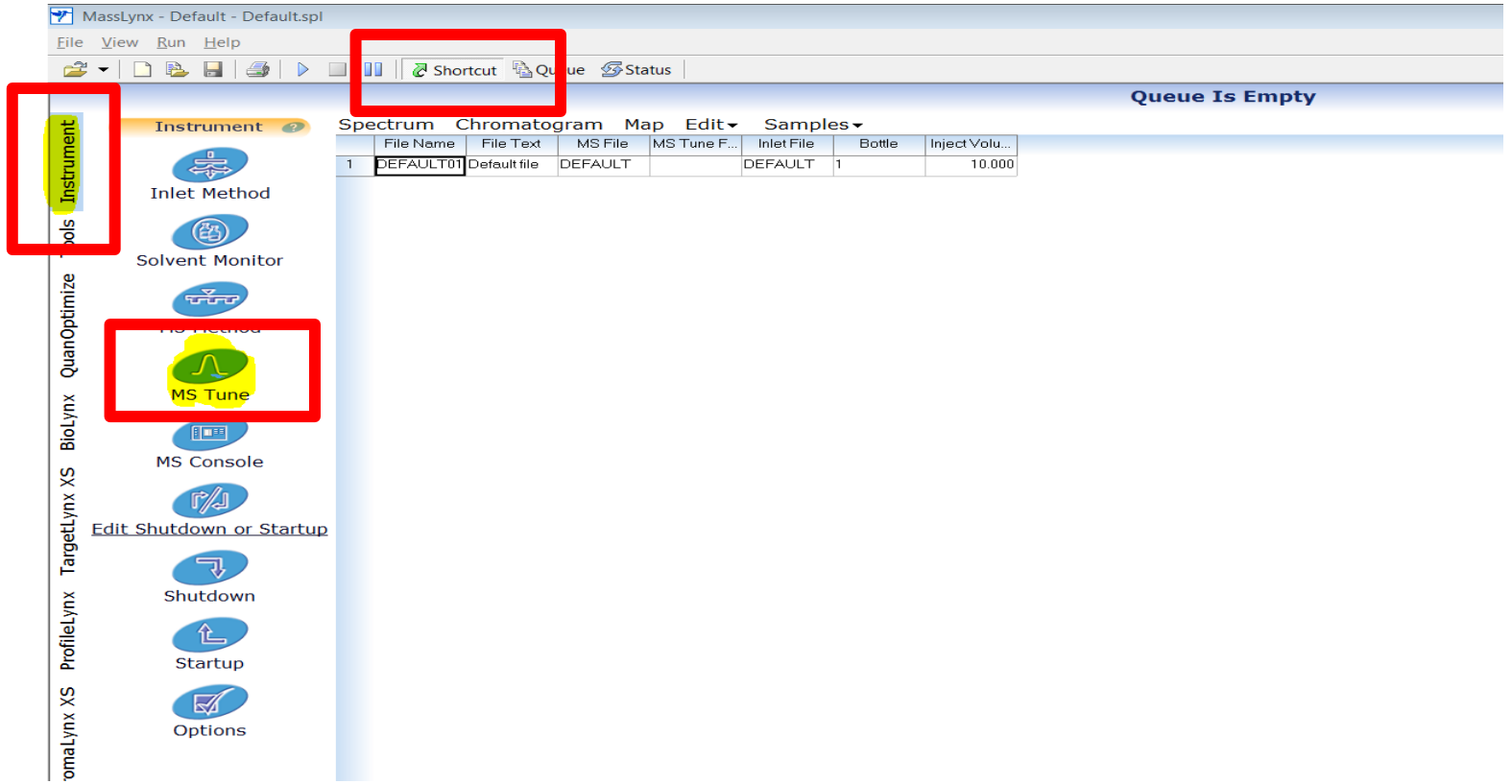
3. Press Start on the right. This will open the IntelliStart wizard (see slide 14).

4. From here it is also possible to switch on the API gas, switch the instrument into Operate and to access the MS Tune page. As an alternative the MS Tune page can also be accessed directly from the MassLynx window (see next slide).

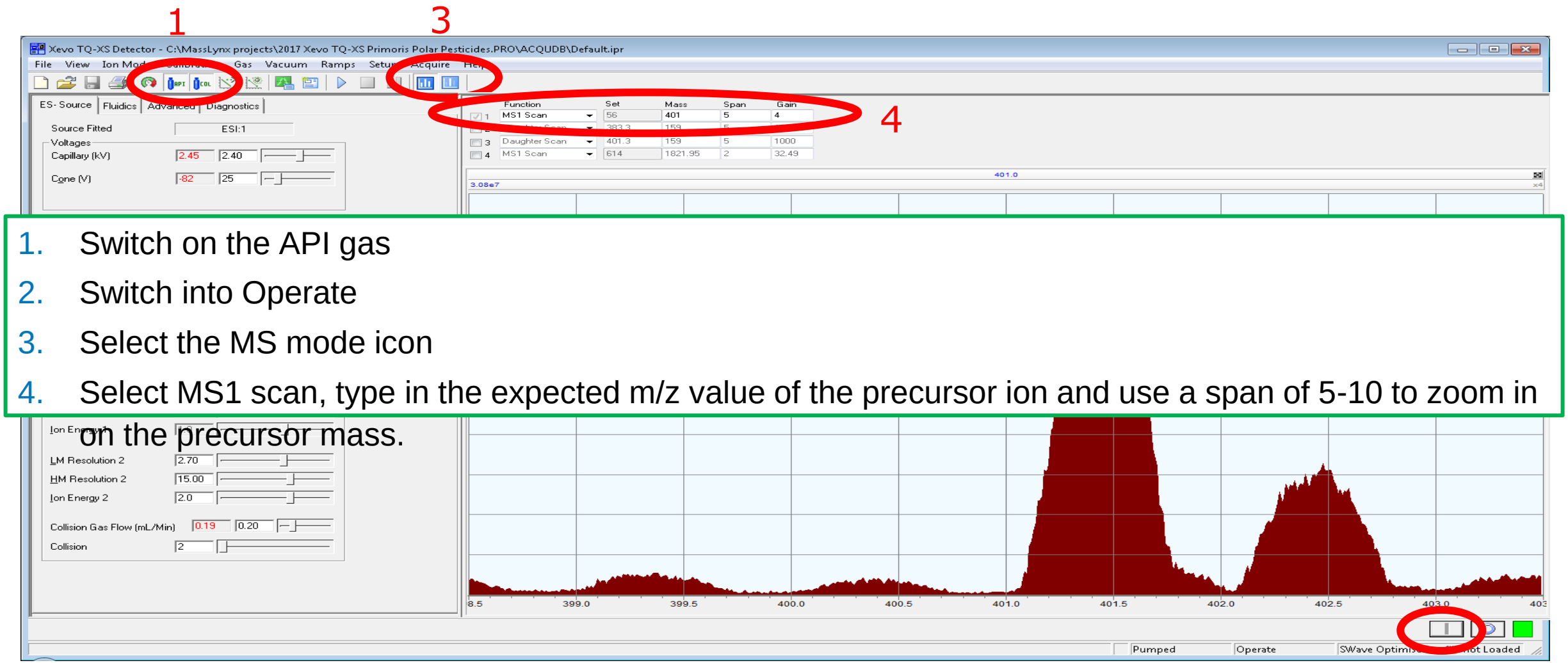


# Access the MS Tune

- In the MassLynx main window, click “Instrument” in the left margin and then click the icon “MS Tune”. Make sure the top toolbar icon is “Shortcut”.



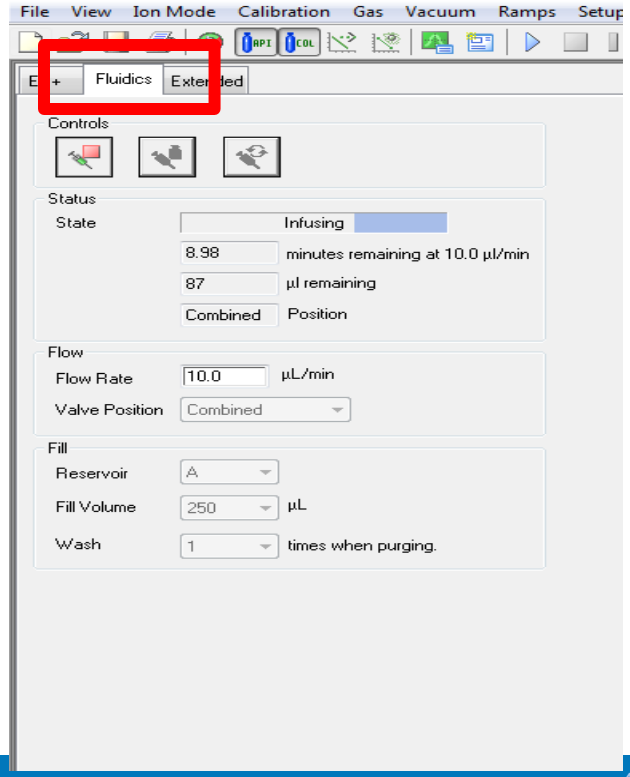
# Switch on the instrument




1. Switch on the API gas
2. Switch into Operate
3. Select the MS mode icon
4. Select MS1 scan, type in the expected m/z value of the precursor ion and use a span of 5-10 to zoom in on the precursor mass.

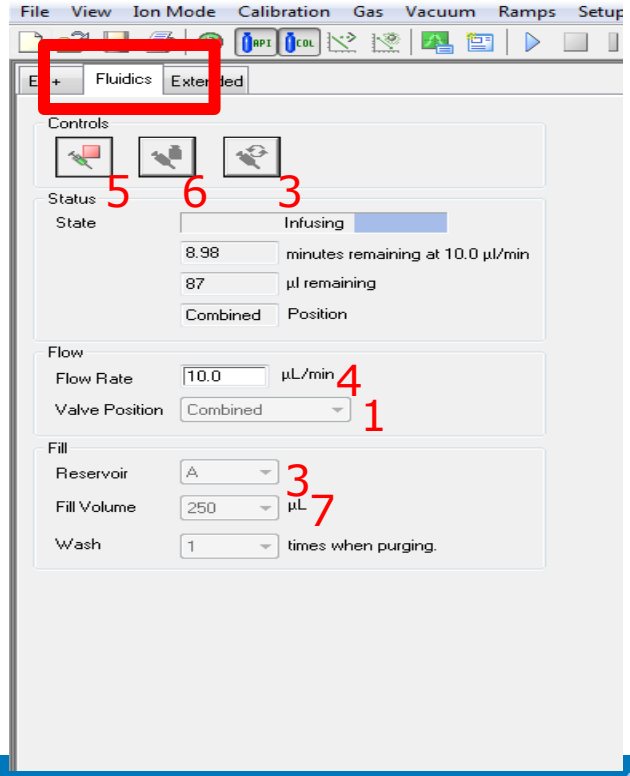
# Fluidics Tab: preparation

- In the **MS Tune** page go to the **Fluidics** tab.
- Make sure the syringe is free from air bubbles. Therefore purge 2-3x with **Wash** solvent prior to first infusion. This will garantuee a stable signal in the spectrum window.
- Prepare a solution of 100 ng/mL in an appropriate solvent (e.g. methanol, acetonitrile).



# Fluidics Tab: operation

1. Select **Combined** mode
2. Set a mobile phase flow rate of 400 µL/min (access from either the MS Console or Inlet Method)
   - Set 80A/20B for early eluters
   - Set 20A/80B for late eluters
   - Set 50A/50B if retention time is not known yet
3. Purge compound prior to infusion from either **reservoir A or B**
4. Set a **Flow Rate** of **10-20 µL/min**
5. Press the **Start** button
6. If the vial is empty, refill it using the **Refill** button
7. If you have limited volume of sample, use less **Fill Volume**



## 2. Run the IntelliStart process

- Ionisation
- IntelliStart wizard
- IntelliStart report

# Ionisation

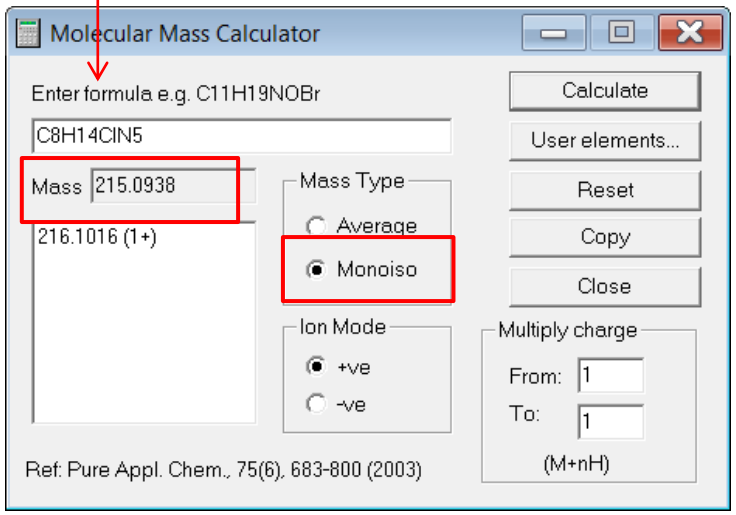
- The goal of the IntelliStart process is to optimize the parent ion (or precursor ion) m/z and corresponding cone voltage in a first stage and a number of daughter ion (or product ion) m/z values with corresponding collision energies.

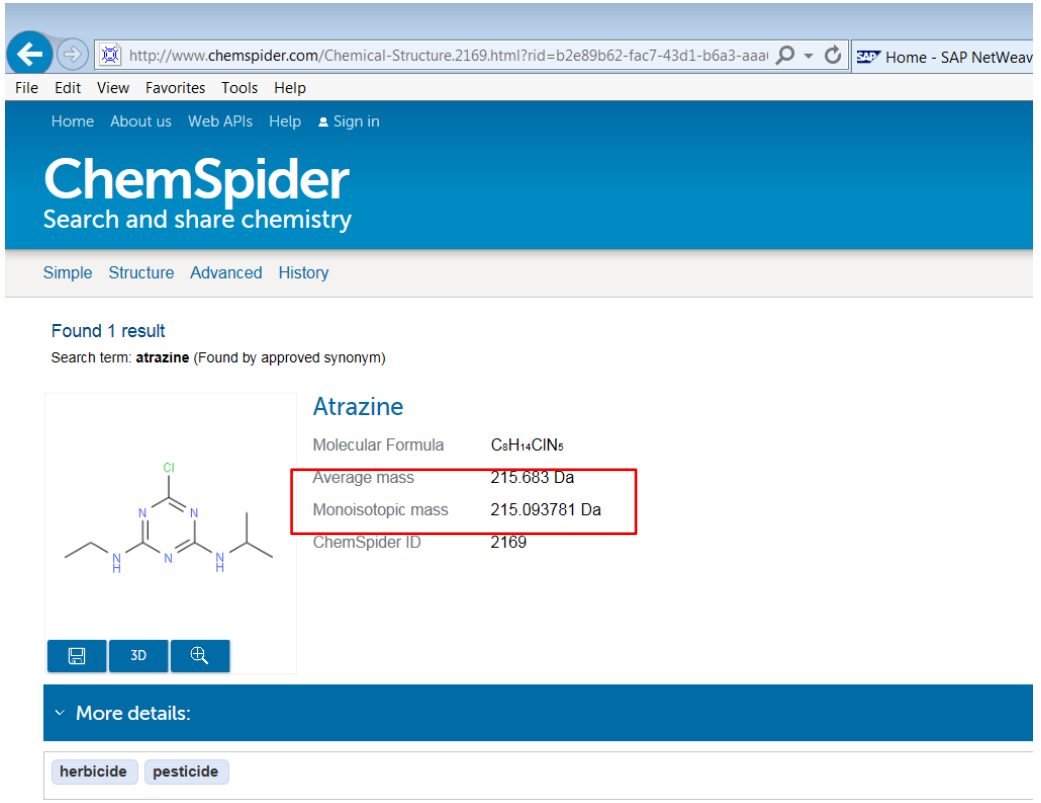
- Use Chemspider.com, other internet tools or literature to determine the compound monoisotopic mass [M] (see next slide).

# Chemspider and MassLynx MM Calculator

- Chemspider provides a formula, average mass and monoisotopic mass for any given name (see right).

- As an alternative a formula can be entered into the MassLynx Molecular Mass Calculator (see below). Access this windows via tools icon in the left margin of the MassLynx main window.



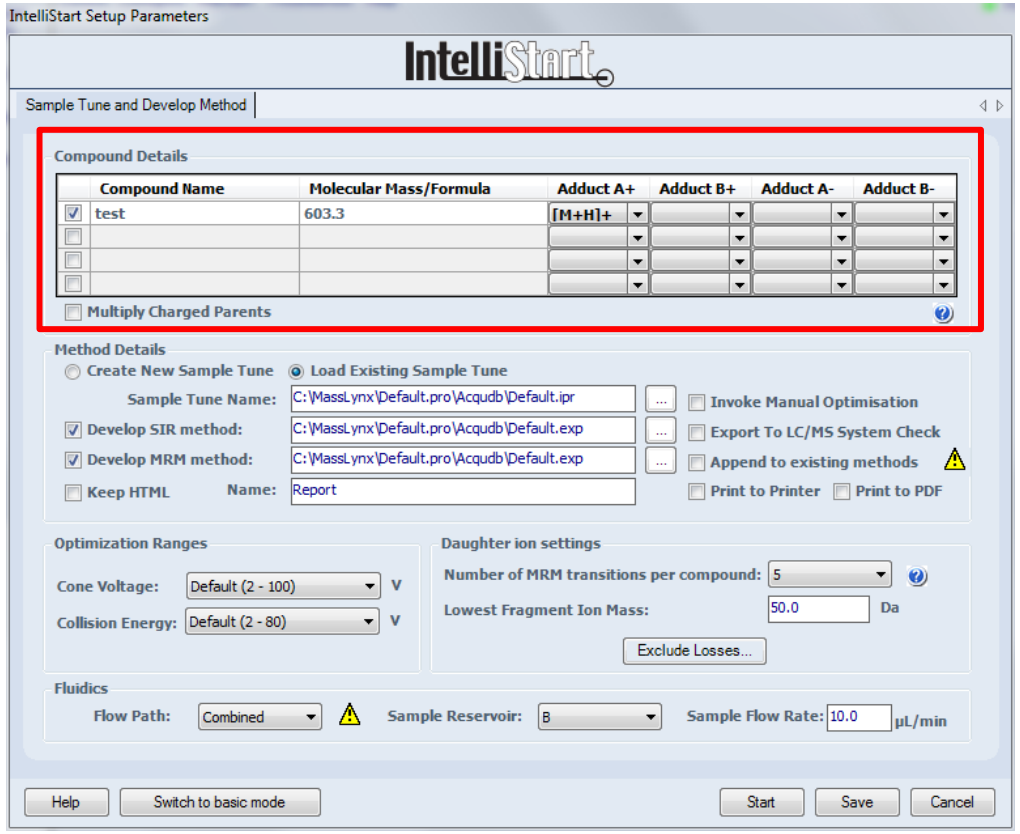



# Parent Ions to be investigated

- In general ES+ provides $[M+H]^+$ = M+1 and ES- results into $[M-H]^-$ = M-1.
- Be aware of the following exceptions, so check for alternative m/z values:
  - Certain ES+ compounds prefer adduct formation:
    - $[M+NH_4]^+$ = M+18
    - $[M+Na]^+$ = M+23
    - $[M+K]^+$ = M+39
  - Certain ES- compounds prefer adduct formation:
    - $[M+Cl]^-$ = M+35
    - $[M+acetate]^-$ or $[M+formate]^-$ = M+45 or M+59
    - $[M+sodium\ formate-H]^-$ or $[M+sodium\ acetate-H]^-$ = M+67 or M+81
  - Certain ES+ compounds fragment easily in the source with loss of:
    - $H_2O$ = M-18
    - MeOH = M-32
    - EtOH = M-46

# IntelliStart wizard

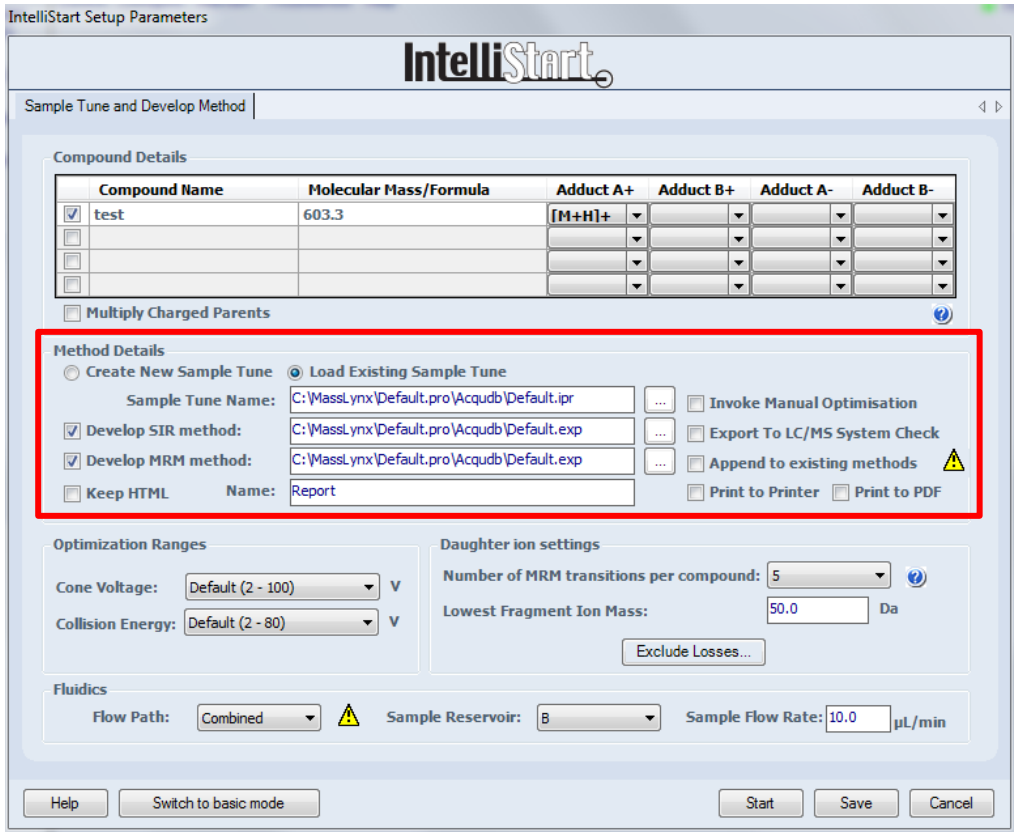
1. Compound Details:
   - Enter up to 4 Compound Names
   - Enter either the Monoisotopic Mass or the Molecular Formula
   - Enter the Adduct(s) of choice
   - If your compound contains a multiply charged ion, select Multiply Charged Parents, and select the ion mode of operation for each compound in the Ion Mode column of the Compound Details table.
   - Tip: Multiply charged ions often require different collision energies to fragment. If the fragments produced are singly charged, they can be of a higher m/z than the precursor ion. Selecting the Multiply Charged Parents check box sets the collision energy and mass scan parameters accordingly.




1

# IntelliStart wizard

2. Method Details – recommended settings:
   - Use the “Load Existing Sample Tune”: IntelliStart will use the information from this MS tune file directly and create an MS method with transitions, optimized cone voltages/collision energies.  To load an existing tune page, of course a tune page needs to be created first. Apply the proper Resolution settings and Ion Energies and set the desolvation temperature to 650C and the desolvation gas flow to 1000 L/hr. Do this both in ESI+ and ESI- mode.
   - Do not use “Invoke Manual Optimization
   - Either optimize for SIR or for MRM. The new MS method will be saved under the file name entered.
   - You can append the MRM data to an existing method to add more compounds using the sample tune function.
   - You can save the report and print it. Avoid special characters for the report name;

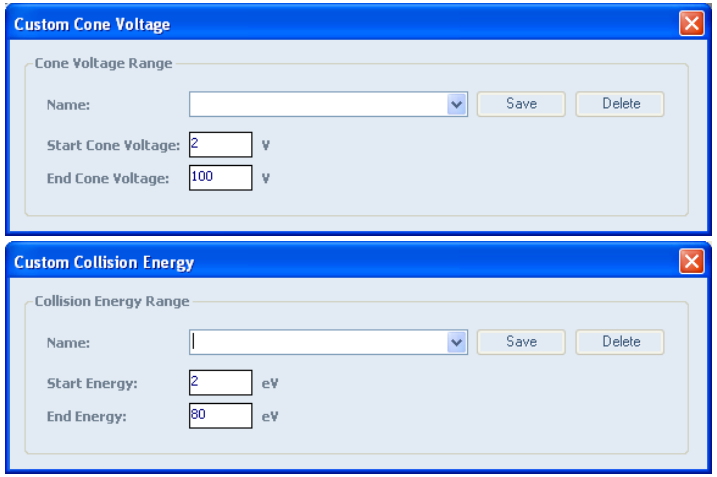



2

# IntelliStart wizard

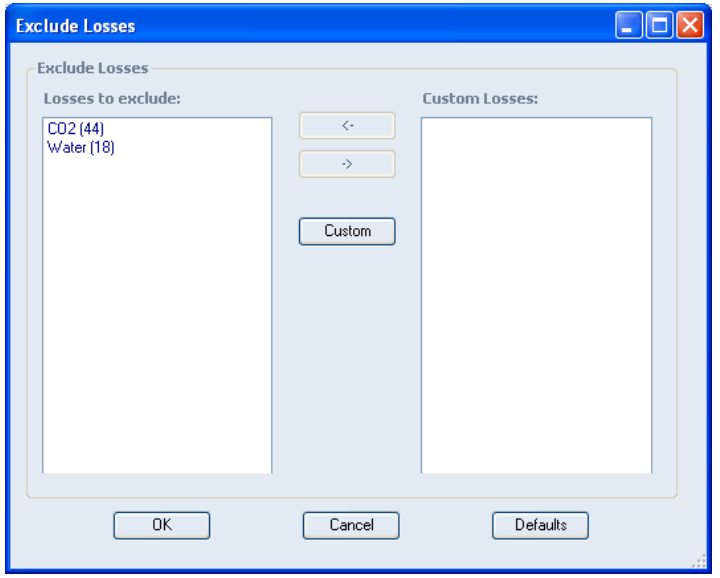
3. Optimization ranges:
   - A custom range for the cone voltage and the collision energy can be configured and saved. This is however not to be recommended. Click the drop down menu to access the editor. Enter new values and new name. Click Save.

3




4. Daughter Ion Settings:
   - For MRM methods, enter how many transitions are to be determined.
   - A setting is available to specify the lowest fragment ion that may be used for the transitions.
   - Exclude losses allows you to exclude possible non-specific fragmentations. Move the losses to exclude to the left (it is recommended to keep the column on the left empty).

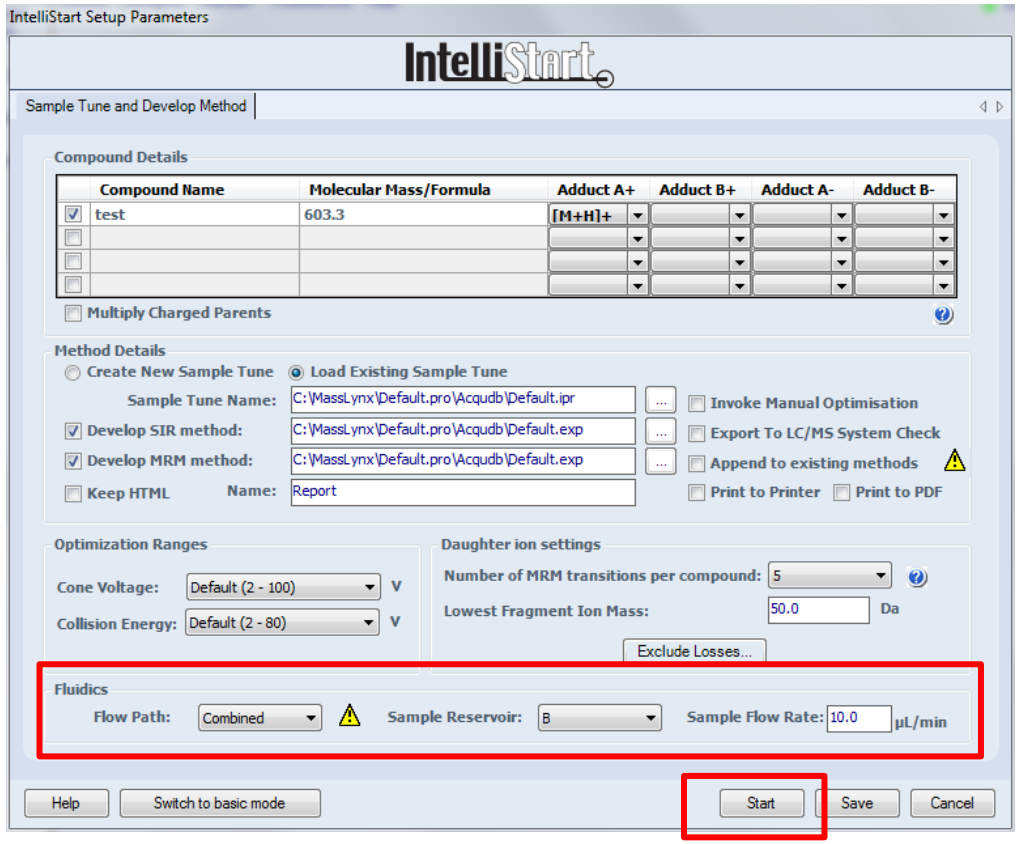
4



# IntelliStart wizard

5. Fluidics
   - Select Combined and make sure a proper flow rate has been selected on the inlet
   - Select the reservoir of choice
   - Select a proper infusion flow rate (of at least 10 µL/min)

6. Click Start to begin
   - Make sure you observe a clear signal on the parent ion m/z in the tune page before you start the IntelliStart optimization

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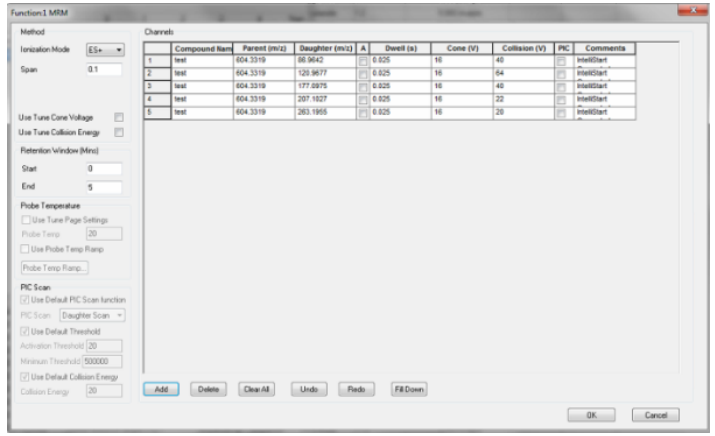
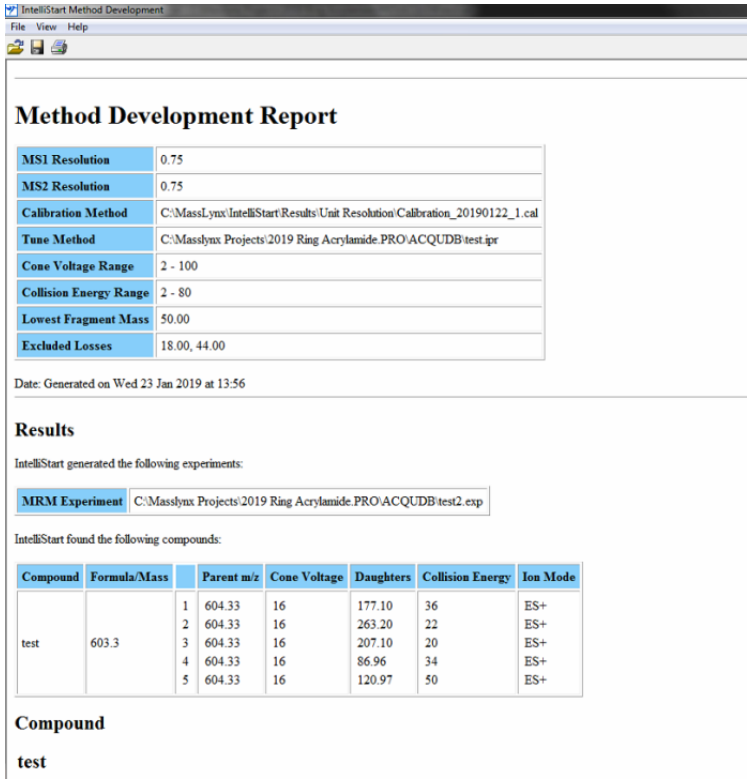
6

# IntelliStart result

- The first test is a comparison of normal ionization mode and soft ionization mode. The process continues with the best ionization mode. Then the IntelliStart optimizes the parent ion and cone voltage. Then it switches to MSMS and searches and centroids the predefined number of product ions.
- At the end of the process, a report is generated showing a summarizing table, MS and MSMS spectra and CV and CE profiles.
- An MRM method is also created with default settings:
  - 5 minute run time (timed functions can be set later during chromatography)
  - 0.1 Da span (set this back to 0)
  - 0.025 s dwell time (please apply autodwell)



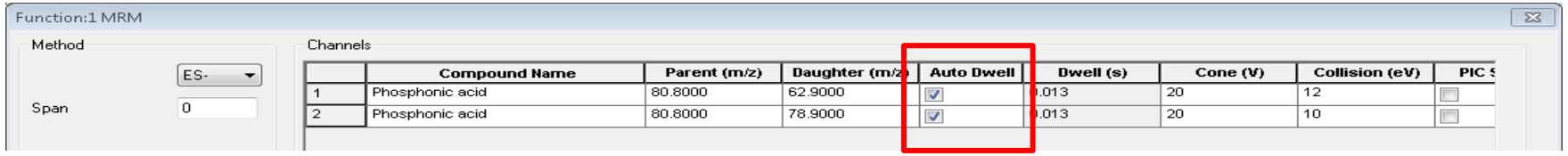
# Important remarks

- At the end of an IntelliStart optimization process, a default tune page from the IntelliStart.pro project is automatically opened. Be aware of this.
- Intellistart may choose a cone voltage which is too low. Please check the plots in the report and make the necessary modifications (see previous slides on manual optimization for more information about this).
- It has been observed that the first IntelliStart optimization process does not always automatically generate an MS method. In that case repeat the process.

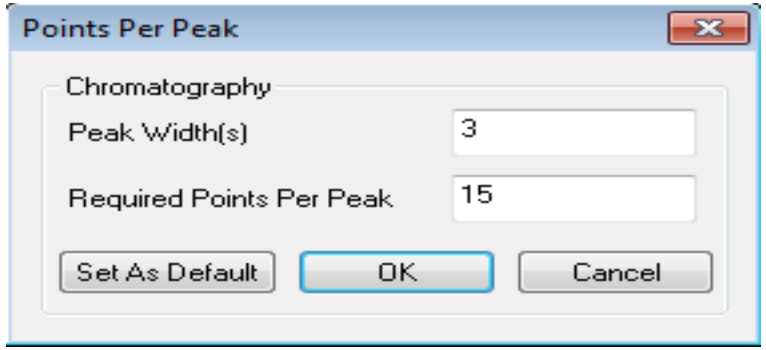
# Additional remarks regarding the MS Method

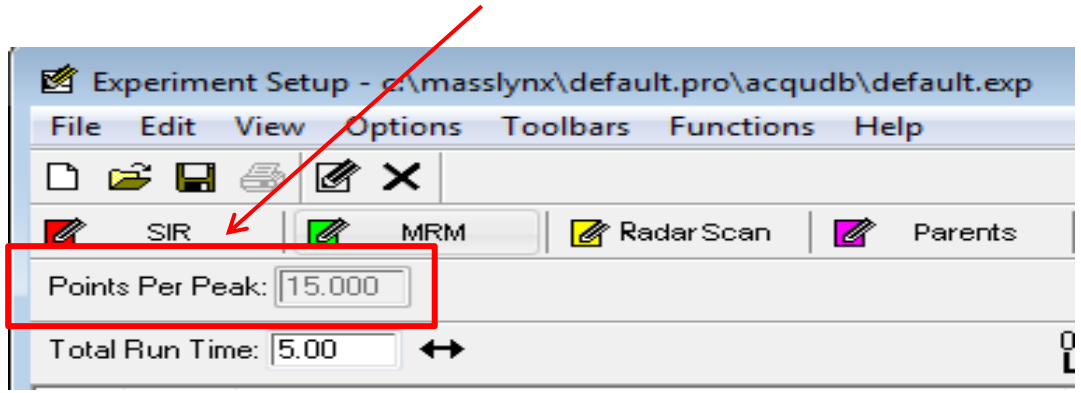
# Auto dwell

- Select auto dwell for an automatic calculation of the dwell time depending on the number of simultaneous transitions (and inter-scan delay times, polarity switching) and the specified points per peak.




- When using the auto dwell option, adjust your peak width and specify +/- 15 points per peak for quantitative analysis (options MS method). The dwell time will be adjusted accordingly. Please verify the actual points per peak of the method.



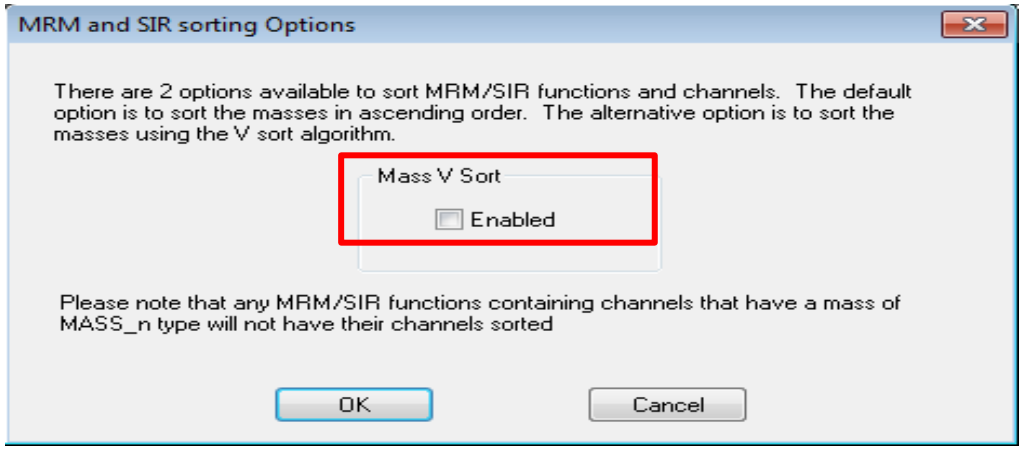



# MRM V-Sort

- There are two options for sorting out the MRM transitions:
    1. acquiring masses in ascending order
    2. V-sort algorithm (acquired in the optimum order of performance, minimizing large voltage jumps)

- This can be important when very short dwell times and inter-scan delay times are used. It is recommended to always use V-sorting as this will never have a negative impact on sensitivity.

- Applying the v-sorting will affect the MS method as it will reorder the functions. It may have an impact on the processing method, though, when the acquisition function numbers are set to 0 there will be no problem.

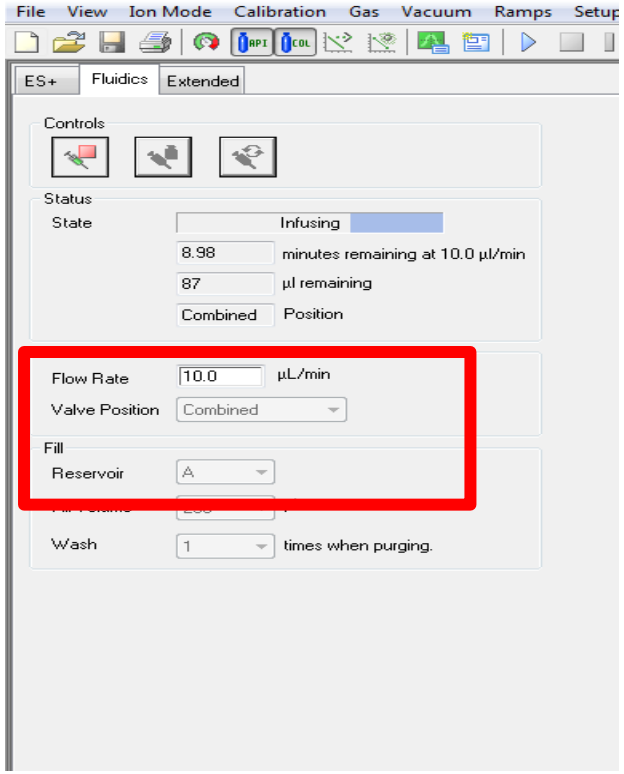


# 3. Shutdown the instrument

- Clean the fluidics
- Switch off the instrument

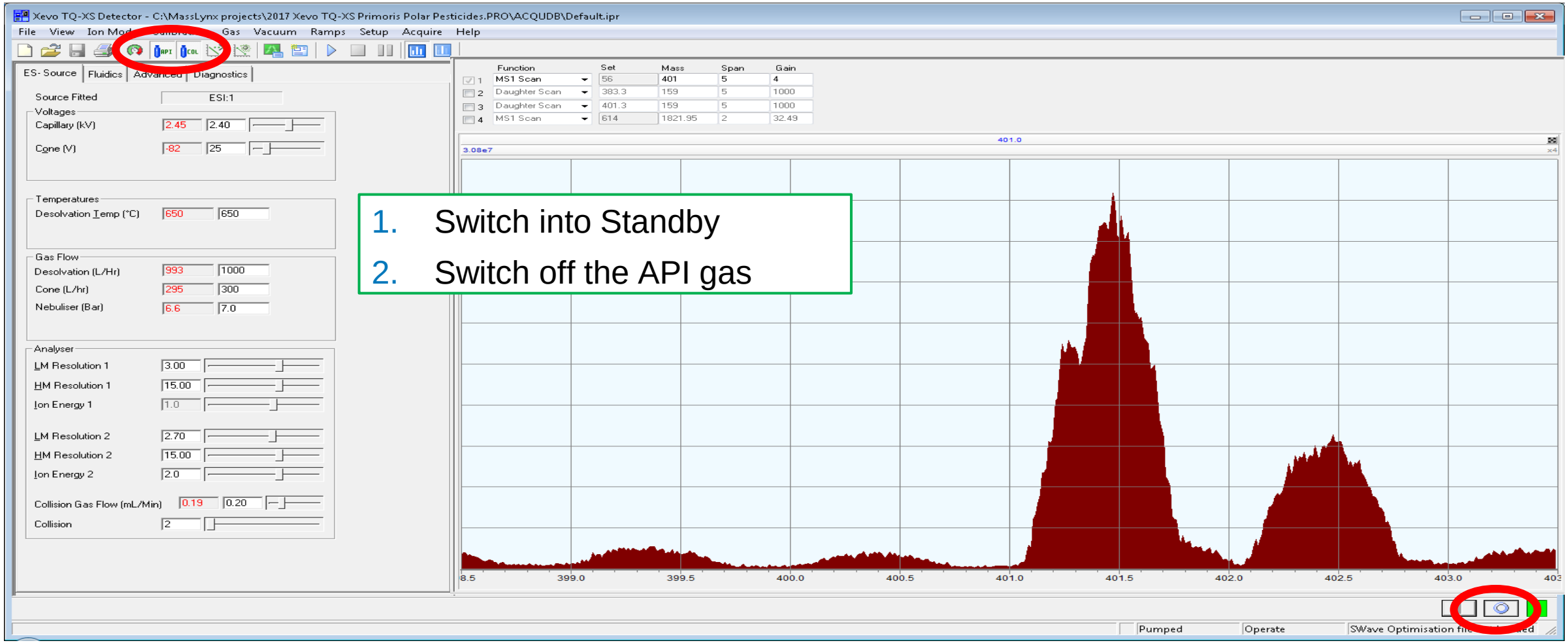
# Fluidics Tab

- In the MS Tune page go back to the Fluidics tab. Replace the active reservoir by a bottle of pure methanol and purge to prevent blockage of the valve and to keep the fluidics system clean.
- After purging, press start in the combined position to flush the valve internal connections & tubing. Keep the flow on for 30s.
- Stop the flow. Leave the bottle of methanol in the actual position.
- Stop the LC mobile phase flow.



# Switch off the instrument

2




1. Switch into Standby
2. Switch off the API gas

1