

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

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

Optimization compounds on 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

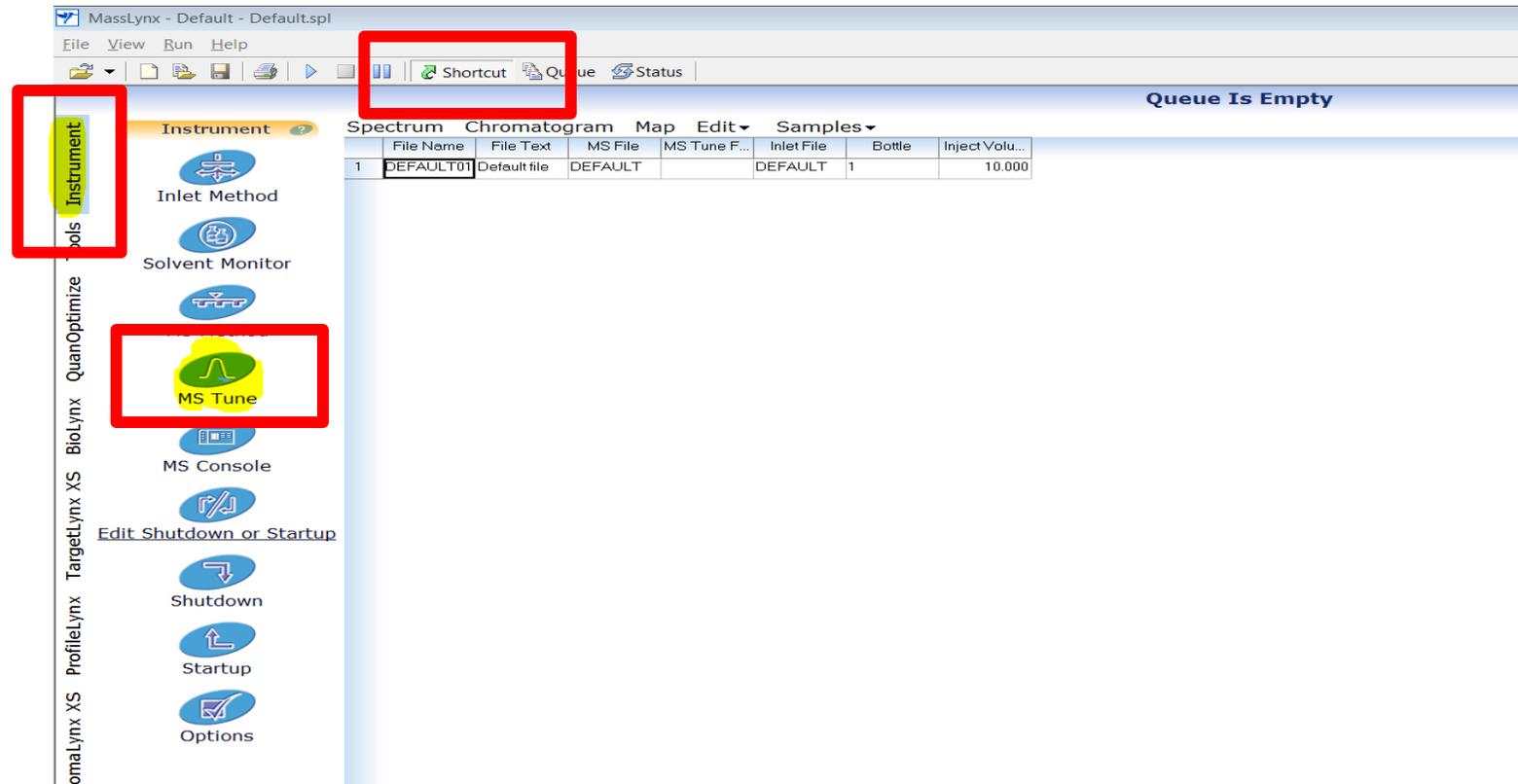
- This presentation serves as a step-by-step guide for manual compound optimization.
- First the steps necessary to **prepare the instrument** are given.
- Then all steps to perform a manual **compound optimization** are explained in detail.
- Finally, the steps necessary to **shutdown the instrument**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

The screenshot shows the Waters Xevo TQ-Smicio MS Detector software interface. The top menu bar includes File, View, Ion Mode, Source, Gas, Vacuum, Ramps, Setup, and Help. The toolbar contains icons for API, On, and other functions. The left panel shows source parameters: Source Voltages (Capillary: 1.03, 1.00; Cone: 43, 40), Source Temperatures (Desolvation Temp: 400, 400), and Source Gas Flow (Desolvation: 992, 1000; Cone: 50, 50). The right panel shows a table of scan parameters:

| Scan | m/z | Span | Gain | |
|-----------------|-------|---------|------|-------|
| 1 MS1 Scan | 401 | 5 | 4 | |
| 2 Daughter Scan | 383.3 | 5 | 1000 | |
| 3 Daughter Scan | 401.3 | 5 | 1000 | |
| 4 MS1 Scan | 614 | 1821.95 | 2 | 32.49 |

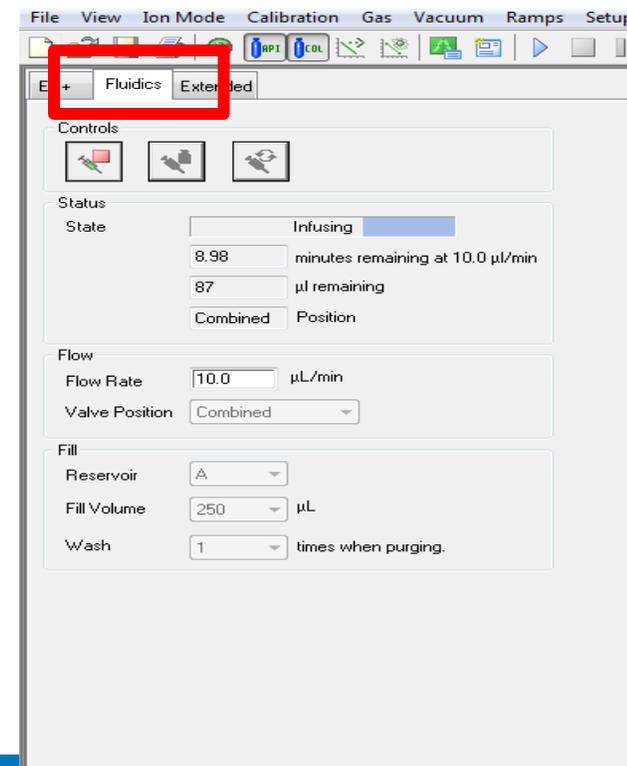
Below the table is a mass spectrum plot showing relative intensity versus m/z. The x-axis ranges from 8.5 to 403.0. A prominent peak is visible at m/z 401.0. The plot is overlaid with a green box containing a list of instructions.

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.

The status bar at the bottom shows 'Ready' and 'Vacuum Ok' buttons. The 'Operate' button is circled in red.

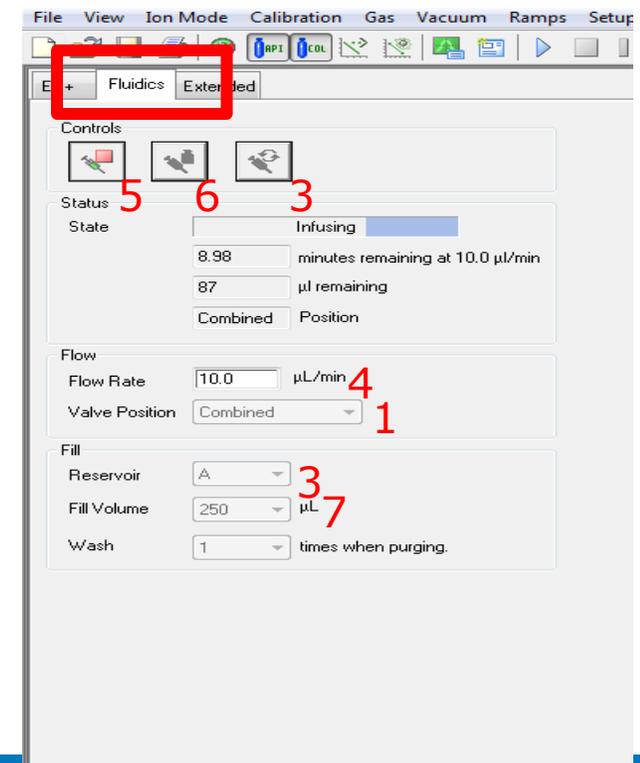
Fluidics Tab: preparation

- 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 guarantee 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 $\mu\text{L}/\text{min}$ (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 $\mu\text{L}/\text{min}$**
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 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.

Molecular Mass Calculator

Enter formula e.g. C11H19NOBr

C8H14ClN5

Mass 215.0938

216.1016 (1+)

Mass Type

Average

Monoiso

Ion Mode

+ve

-ve

Multiply charge

From: 1

To: 1

(M+nH)

Ref: Pure Appl. Chem., 75(6), 683-800 (2003)

Found 1 result

Search term: **atrazine** (Found by approved synonym)

Atrazine

Molecular Formula C₈H₁₄ClN₅

Average mass 215.683 Da

Monoisotopic mass 215.093781 Da

ChemSpider ID 2169

herbicide pesticide

- 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

The screenshot displays the Waters Xevo TQ-Smicro MS Detector software interface. On the left, the 'ES+' panel shows various instrument parameters such as Source Voltages (Capillary at 2.01 kV, Cone at 43 V), Source Temperatures (Desolvation at 400 °C), Source Gas Flow (Desolvation at 993 L/hr, Cone at 50 L/hr), and Analyser settings (LM/HM Resolutions and Ion Energies). The top right features a table of scan functions:

| Function | Set | Mass | Span | Gain |
|-----------------|-------|---------|------|-------|
| 1 MS1 Scan | 56 | 401.4 | 5 | 8 |
| 2 Daughter Scan | 383.3 | 159 | 5 | 1000 |
| 3 Daughter Scan | 401.3 | 159 | 5 | 1000 |
| 4 MS1 Scan | 614 | 1821.95 | 2 | 32.49 |

The main plot area shows a mass spectrum with a prominent peak at 401.4 m/z. A red box highlights the 'Span' and 'Gain' values for the first scan (5 and 8, respectively). A red box with the text 'Select a proper Gain value' points to the Gain column. Another red box with the text 'Or double click here to zoom in' points to the peak at 401.4 m/z. A third red box with the text 'Or double click here to zoom out' points to the x-axis at the bottom of the plot. A fourth red box with the text 'For best practice zoom in on the y-axis till the peak reaches the top of the spectrum window.' points to the y-axis. The status bar at the bottom shows 'Ready' and 'Vacuum Ok Operate'.

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)

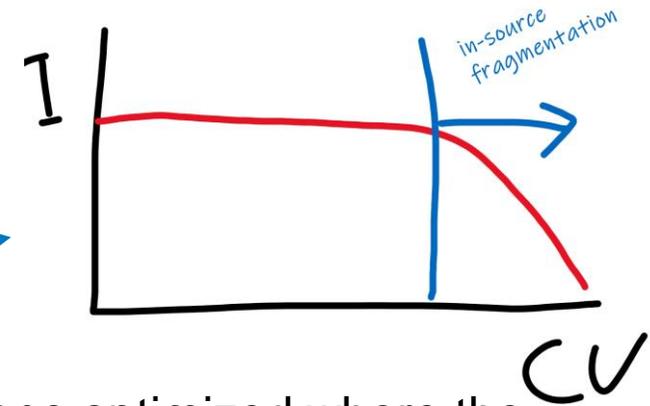
- **Capillary Voltage: voltage needed to send ions to the surface of the charged droplets, needed for efficient transfer of ions into the gas phase**
 - Compound dependent
 - Optimizes between 0.5 and 3 kV
 - Rarely optimizes above 3kV
 - Values above 3kV in ES- may result into a blue discharge on the 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

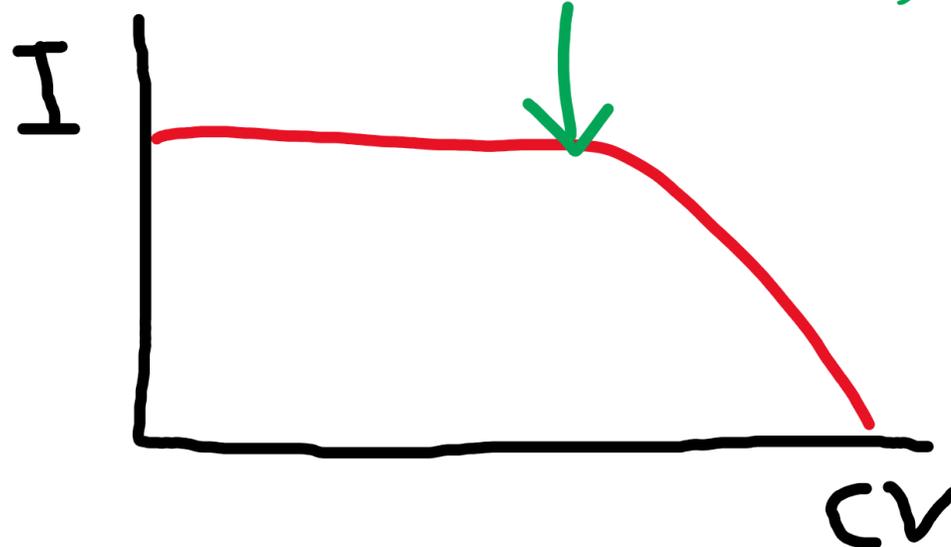
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- **Cone voltage: voltage needed to send ions into the first vacuum region**

- 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.



Optimum cone voltage



- **Desolvation Temperature: temperature of the N₂ 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.

- **Desolvation Gas Flow: N₂ 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 N₂ 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.

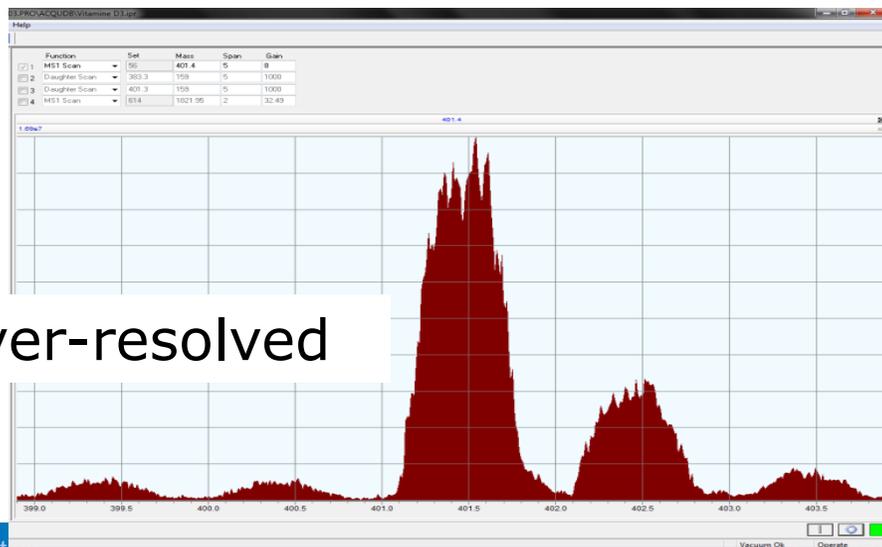
Resolution Settings (not to be tuned!)

LM Resolution 1 and HM Resolution 1

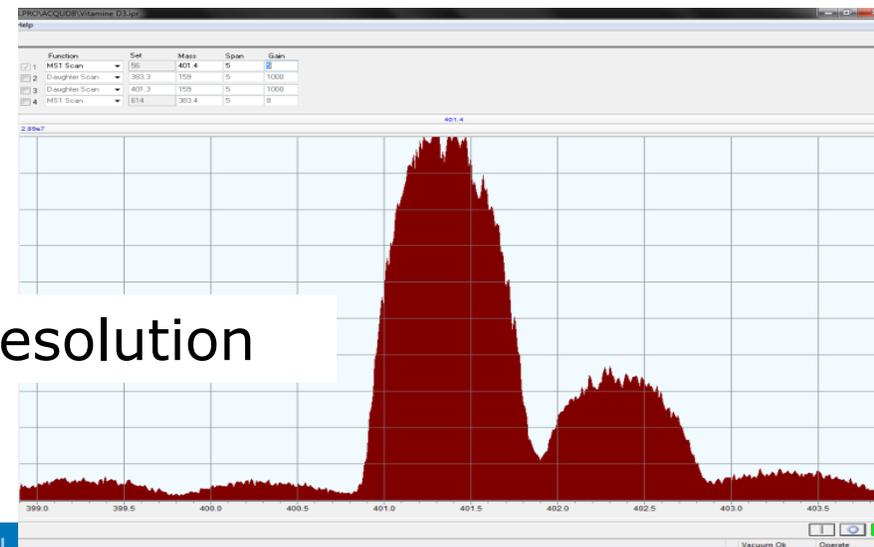
- The resolution settings should be set once a year after PM and must be kept the same for all methods on the same MS. The resolution settings should be provided by a Waters engineer after installation or PM.
- You should check if the resolution settings are OK. Aim at unit resolution, *i.e.* 1 Da at 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

Right: LM Res 15, HM Res 15



Slightly over-resolved



Unit Resolution

Ion Energies (not to be tuned!)

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

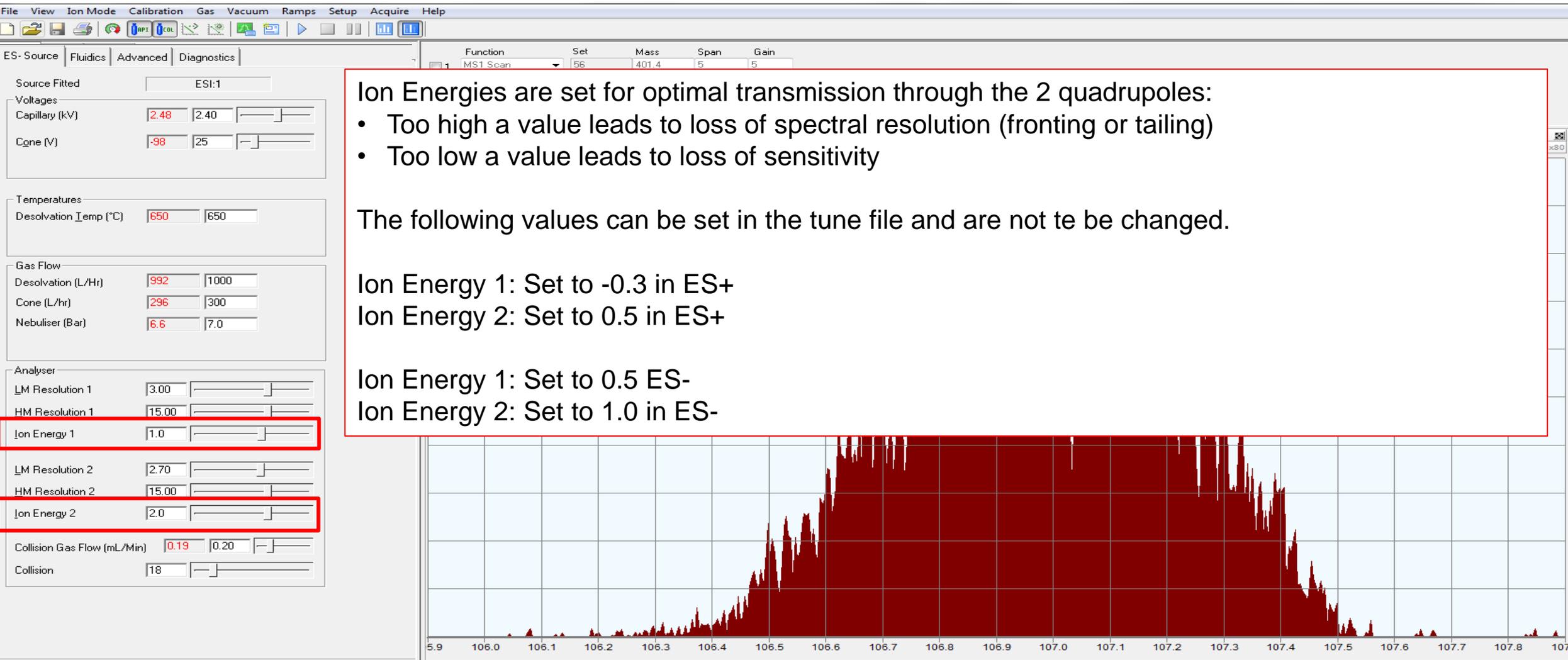
The following values can be set in the tune file and are not to be changed.

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

The screenshot shows the Waters MS2 tune software interface. On the left is a control panel with various parameters. On the right is a table of scan functions and two mass spectra plots. Red circles and numbers 1, 2, and 3 highlight specific elements: 1 points to the 'Scan' button, 2 points to the 'MS2' button, and 3 points to the scan function table. Two text boxes provide instructions for the spectra: 'Spectrum 1: zoom in on a daughter m/z of interest' and 'Spectrum 2: zoom out on the entire expected daughter ion m/z range, keeping the parent m/z visible on the right side'. A red arrow points from the text box to the parent ion peak in Spectrum 2.

| Function | Set | Mass | Span | Gain | |
|----------|---------------|-------|-------|------|----|
| 2 | Daughter Scan | 401.4 | 401.4 | 5 | 20 |
| 3 | Daughter Scan | 401.4 | 210 | 400 | 20 |
| 4 | MS1 Scan | | | | 8 |

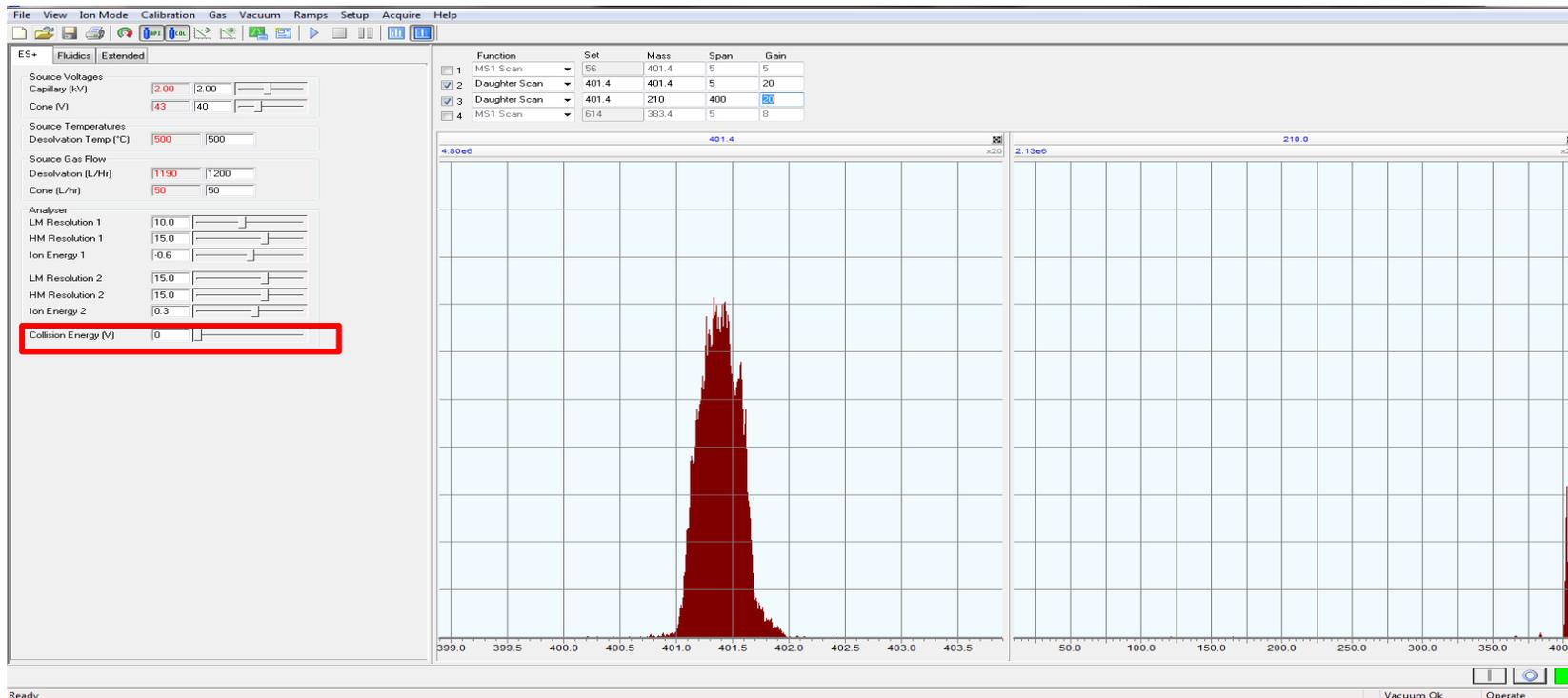
1 (Scan button) **2** (MS2 button) **3** (Scan function table)

Spectrum 1: zoom in on a daughter m/z of interest

Spectrum 2: zoom out on the entire expected daughter ion m/z range, keeping the parent m/z visible on the right side

Step 2: MS2 tune

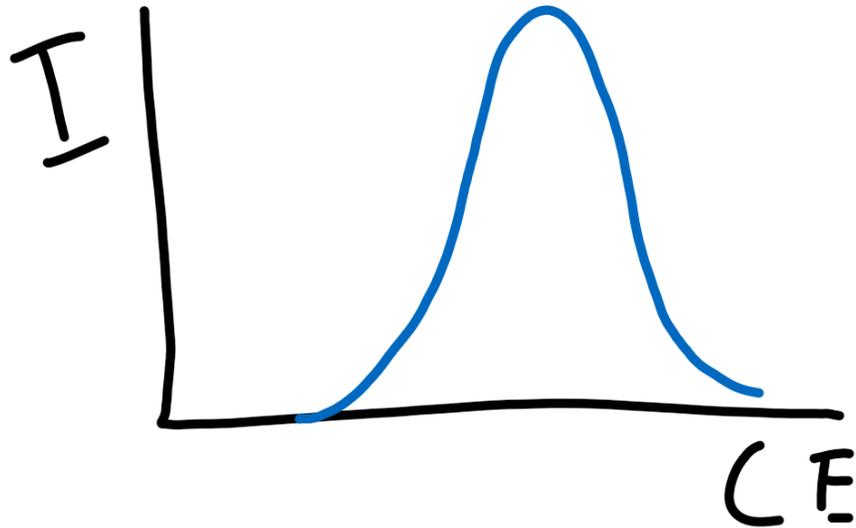
- **HINT:** Start at a very low collision energy (you will predominantly see the parent m/z) and gently increase the energy up to roughly 60eV and see which daughters of interest occur in the right window. Aim at daughters with highest intensity. Using the left window select a daughter m/z of interest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

Optimization collision energy



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

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- 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

The screenshot displays the Waters Xevo TQ-Smicro MS Detector software interface. The main window shows various instrument parameters and a toolbar. A red circle highlights the play button icon in the toolbar, with a red arrow pointing to the Acquisition Setup dialog box. The dialog box is open, showing the following settings:

| Function | Set | Mass | Span | Gain |
|-----------------|-------|-------|------|------|
| 1 MS1 Scan | 56 | 401.4 | 5 | 5 |
| 2 Daughter Scan | 401.4 | 401.4 | 2 | 20 |
| 3 Daughter Scan | 401.4 | 210 | 400 | 20 |

The Acquisition Setup dialog box contains the following fields:

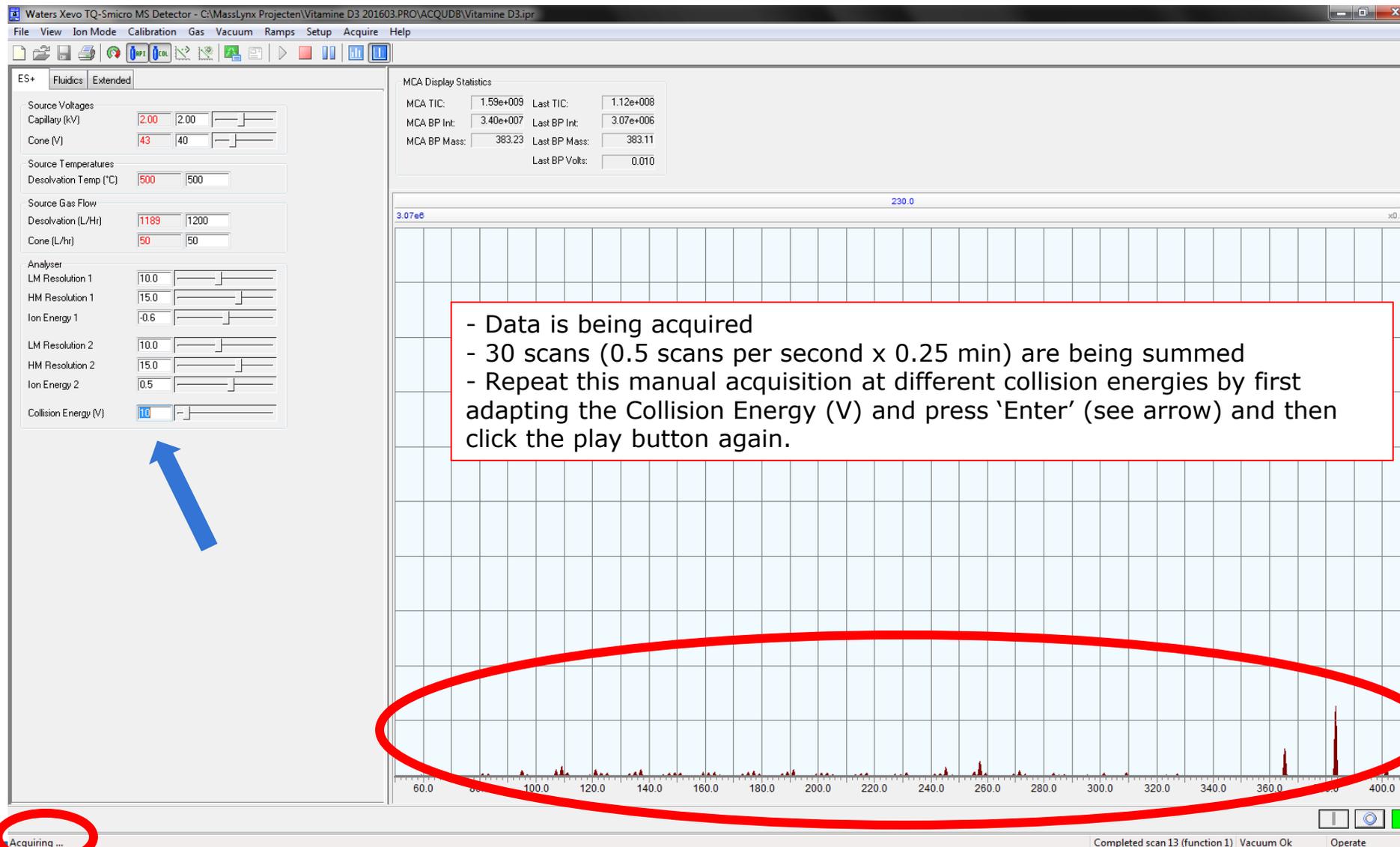
- File: Data File Name: VITAMINE D3 MSMS 05EV, Text: Test
- Data: Function: Daughter Scan, Data Format: MCA, Dual Mode: Ion Mode: ES
- Masses (m/z): Set Mass: 401.4, Start Mass: 50, End Mass: 410
- Time: Run Duration (mins): 0.25, Scan Time (s): 0.5

Buttons: Start, Close, Origin...

The main window displays two mass spectra plots. The left plot shows a broad peak centered around 401.4 m/z. The right plot shows a narrow peak centered around 210.0 m/z. The status bar at the bottom indicates 'Ready' and 'Vacuum Ok Operate'.

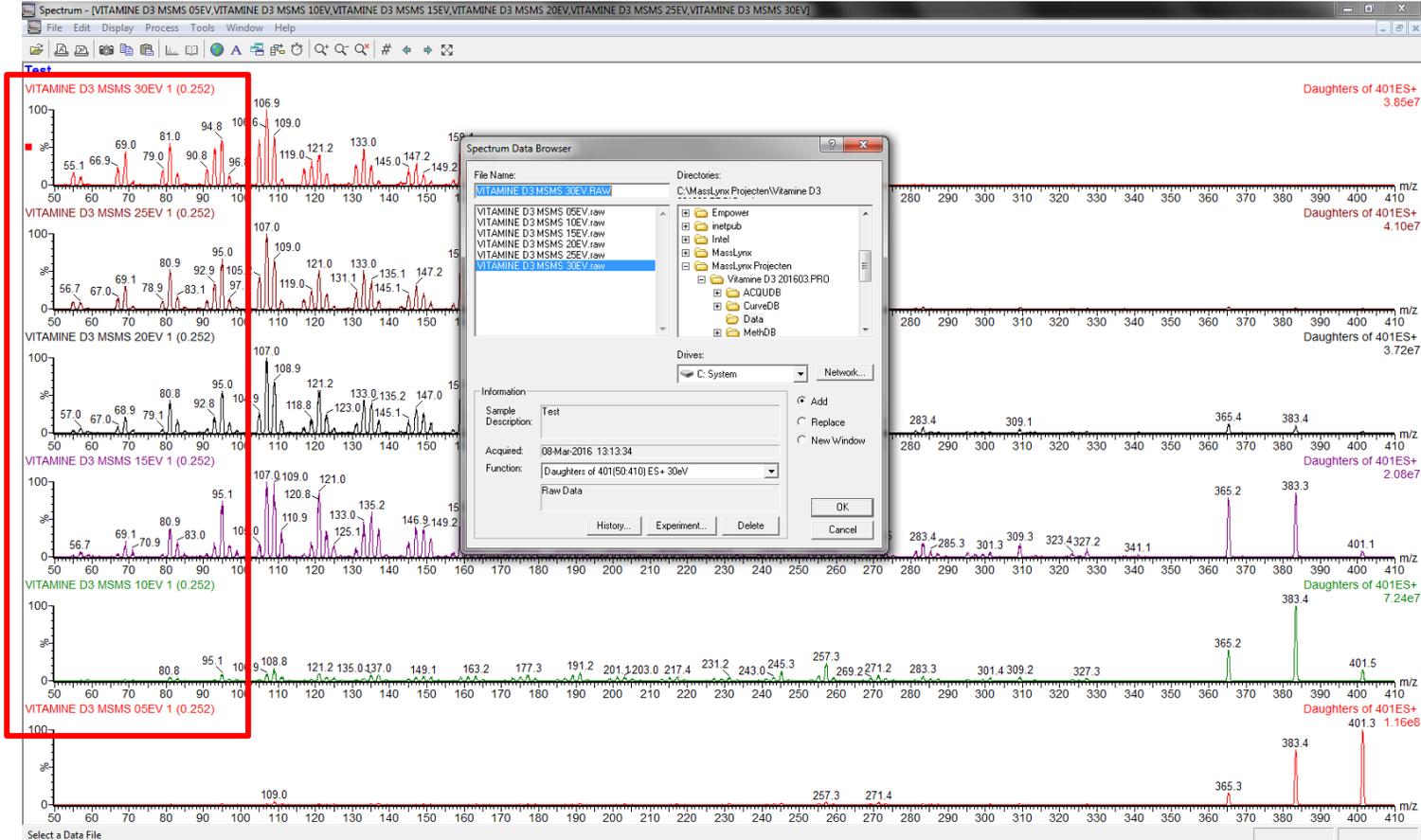
- Select the play button icon from the toolbar
- Give a specific file name
- Select Daughter Scan, MCA
- Select 0.25 of run duration and 0.5 scan time
- Fill in Set Mass, Start Mass and End Mass
- Hit Start

Manual acquisition of daughter ion spectra



Manual acquisition of daughter ion spectra

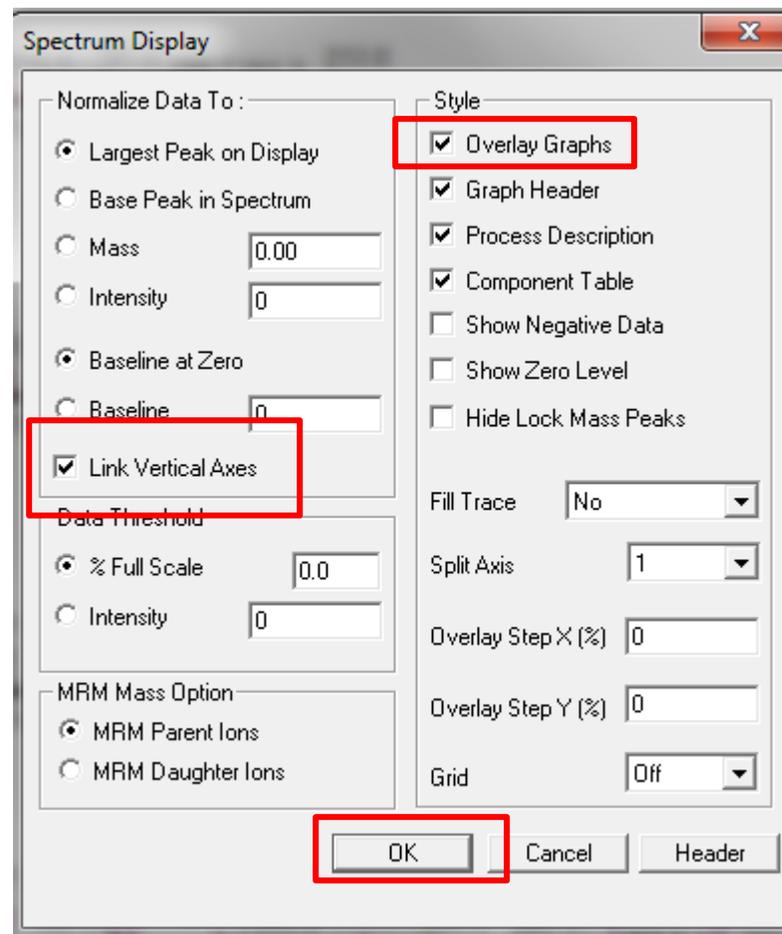
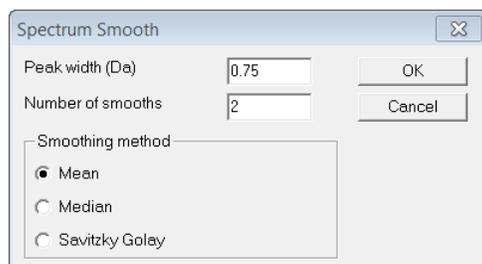
- Go to the sample list and select spectrum from the toolbar
- Open all acquired spectra



Manual acquisition of daughter ion spectra

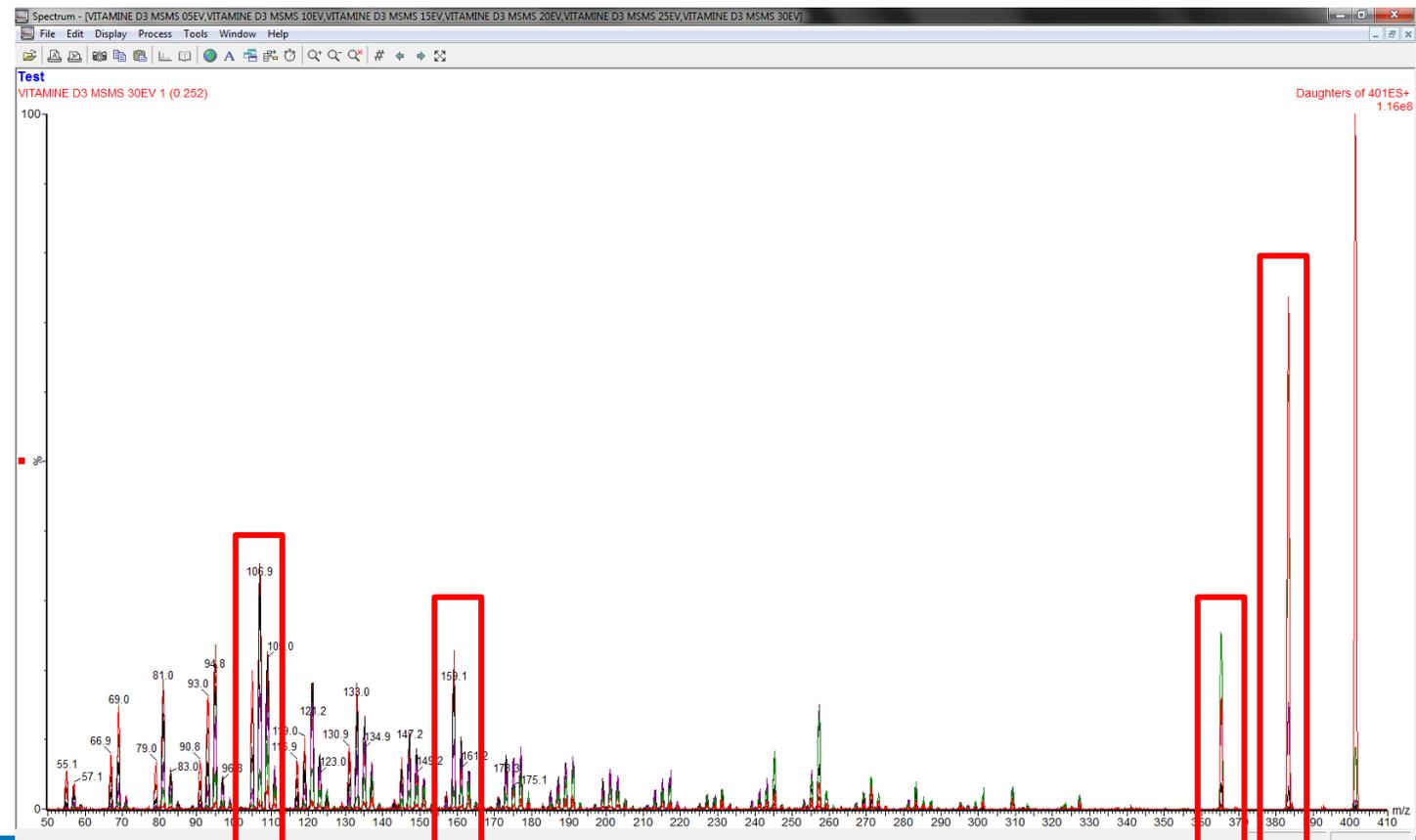
- For best comparison, align and overlay the spectra
- In the spectrum toolbar > display > view
- Select *Link Vertical Axes*
- Select *Overlay Graphs*
- Press *OK*

- 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

- 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.

Function:1 MRM

Method

Ionization Mode ES+

Span

Use Tune Cone Voltage

Use Tune Collision Energy

Retention Window (Mins)

Start

End

Probe Temperature

Use Tune Page Settings

Probe Temp

Use Probe Temp Ramp

Probe Temp Ramp...

PIC Scan

Use Default PIC Scan function

PIC Scan Daughter Scan

Use Default Threshold

Activation Threshold

Minimum Threshold

Use Default Collision Energy

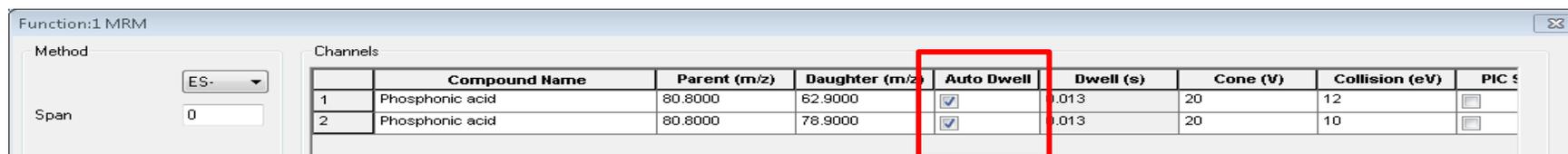
Collision Energy

| Compound Name | Parent (m/z) | Daughter (m/z) | A | Dwell (s) | Cone (V) | Collision (V) | PIC | Comments |
|---------------|--------------|----------------|-------------------------------------|-----------|----------|---------------|--------------------------|----------|
| TnBP | 267.2500 | 98.8500 | <input checked="" type="checkbox"/> | 0.122 | 40 | 18 | <input type="checkbox"/> | |

Add Delete Clear All Undo Redo Fill Down

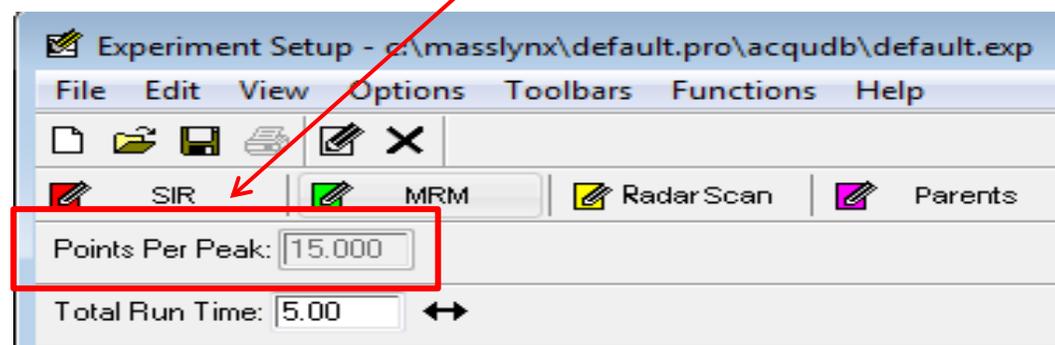
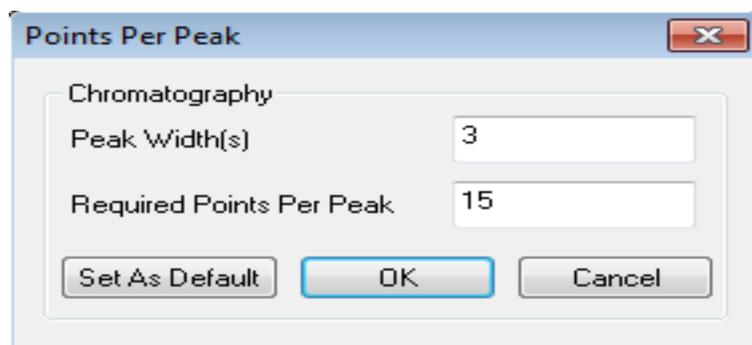
OK Cancel

- 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.

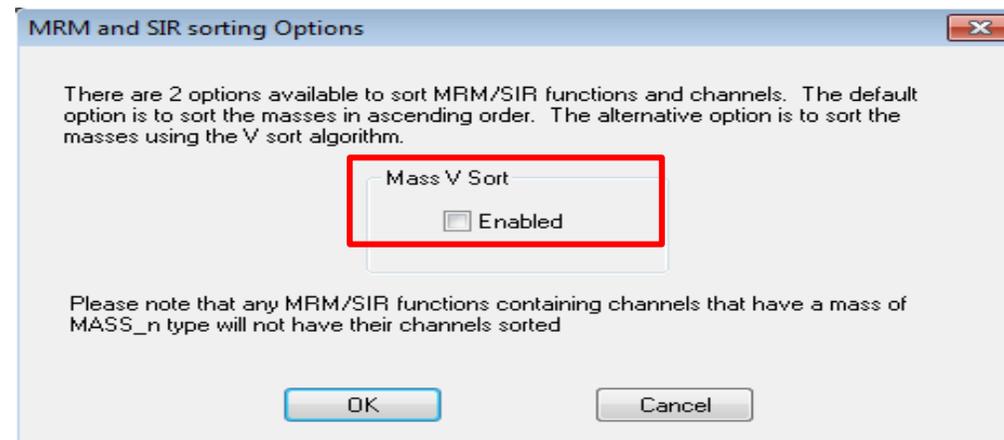


| | Compound Name | Parent (m/z) | Daughter (m/z) | Auto Dwell | Dwell (s) | Cone (V) | Collision (eV) | PIC |
|---|-----------------|--------------|----------------|-------------------------------------|-----------|----------|----------------|--------------------------|
| 1 | Phosphonic acid | 80.8000 | 62.9000 | <input checked="" type="checkbox"/> | 0.013 | 20 | 12 | <input type="checkbox"/> |
| 2 | Phosphonic acid | 80.8000 | 78.9000 | <input checked="" type="checkbox"/> | 0.013 | 20 | 10 | <input type="checkbox"/> |

- 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.



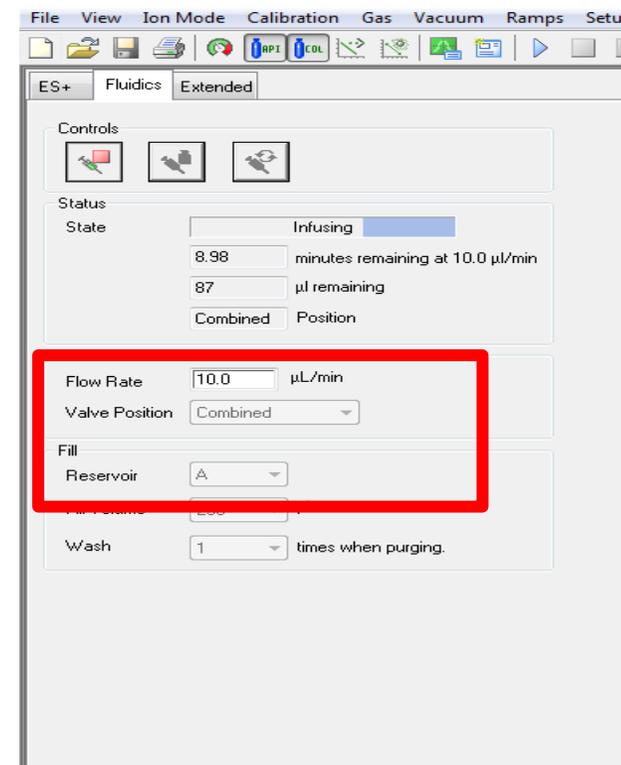
- There are two options for sorting out the MRM transitions:
 1. acquiring masses in ascending order
 2. V-sort algorithm (acquired in the optimum order of performance, minimizing large voltage jumps)
- This can be important when very short dwell times and inter-scan delay times are used. It is recommended 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**

- 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

ES- Source | Fluidics | Advanced | Diagnostics

Source Fitted: ESI:1

Voltages

Capillary (kV): 2.45 | 2.40

Cone (V): -82 | 25

Temperatures

Desolvation Temp (°C): 650 | 650

Gas Flow

Desolvation (L/Hr): 993 | 1000

Cone (L/hr): 295 | 300

Nebuliser (Bar): 6.6 | 7.0

Analyser

LM Resolution 1: 3.00

HM Resolution 1: 15.00

Ion Energy 1: 1.0

LM Resolution 2: 2.70

HM Resolution 2: 15.00

Ion Energy 2: 2.0

Collision Gas Flow (mL/Min): 0.19 | 0.20

Collision: 2

| Function | Set | Mass | Span | Gain |
|-----------------|-------|---------|------|-------|
| 1 MS1 Scan | 56 | 401 | 5 | 4 |
| 2 Daughter Scan | 383.3 | 159 | 5 | 1000 |
| 3 Daughter Scan | 401.3 | 159 | 5 | 1000 |
| 4 MS1 Scan | 614 | 1821.95 | 2 | 32.49 |

3.08e7 401.0

8.5 399.0 399.5 400.0 400.5 401.0 401.5 402.0 402.5 403.0 403.5

Pumped Operate SWave Optimisation file saved

1

Intellistart optimization

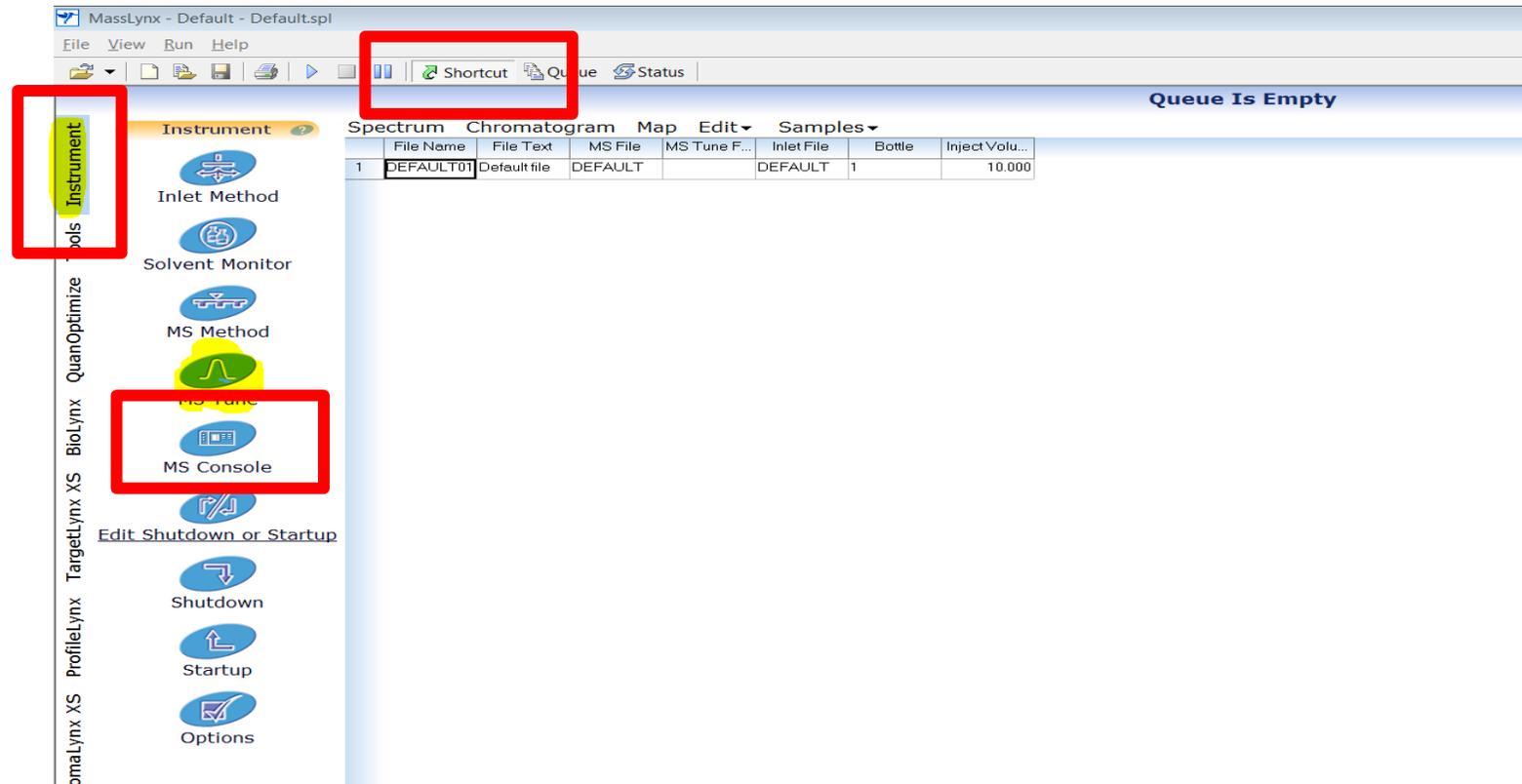
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- First the steps necessary to **prepare the instrument** are given.
- Then all steps to perform an IntelliStart automatic **compound optimization** are explained in detail.
- Finally, the steps necessary to **shutdown the instrument** are highlighted.

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”.



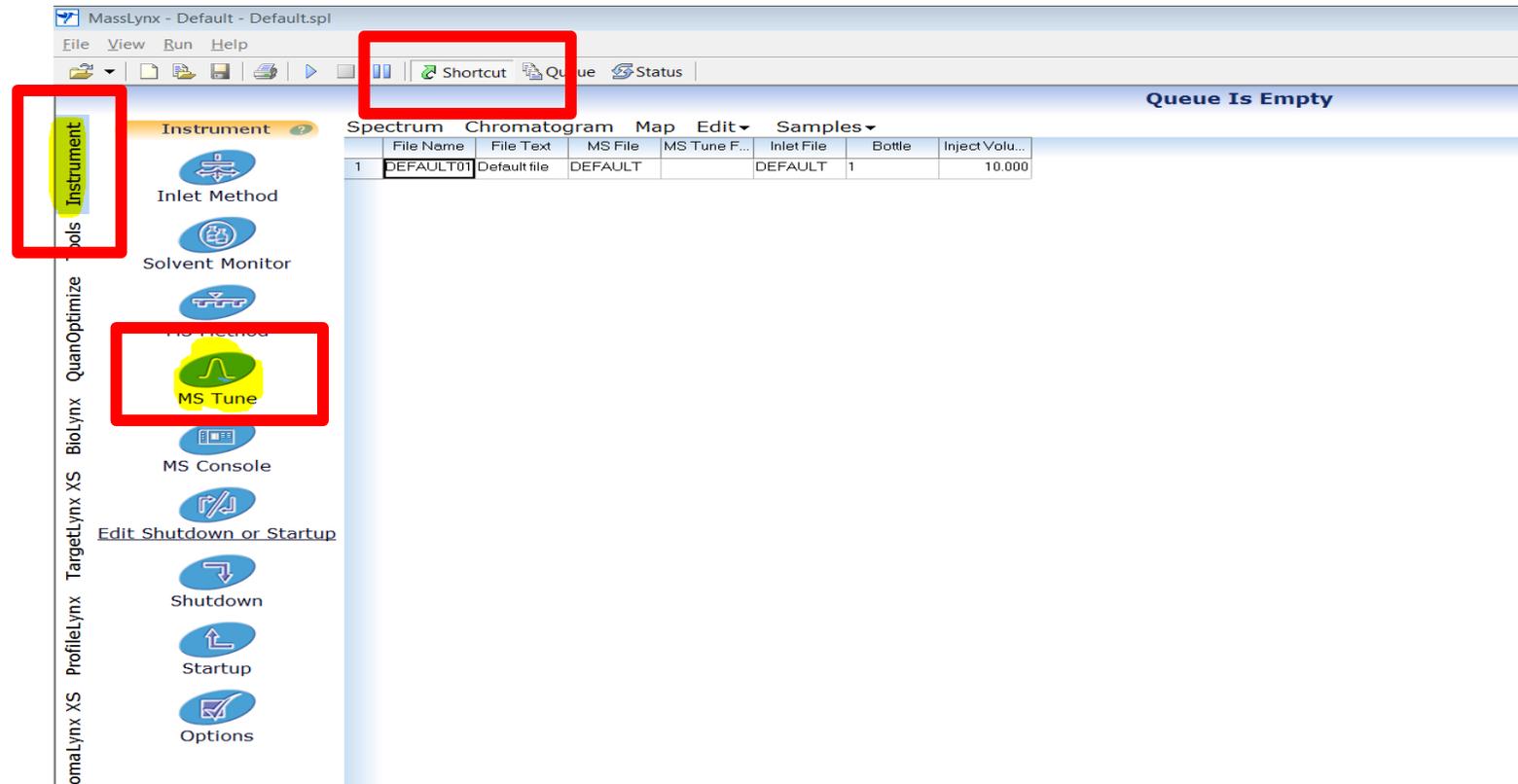
Console

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.

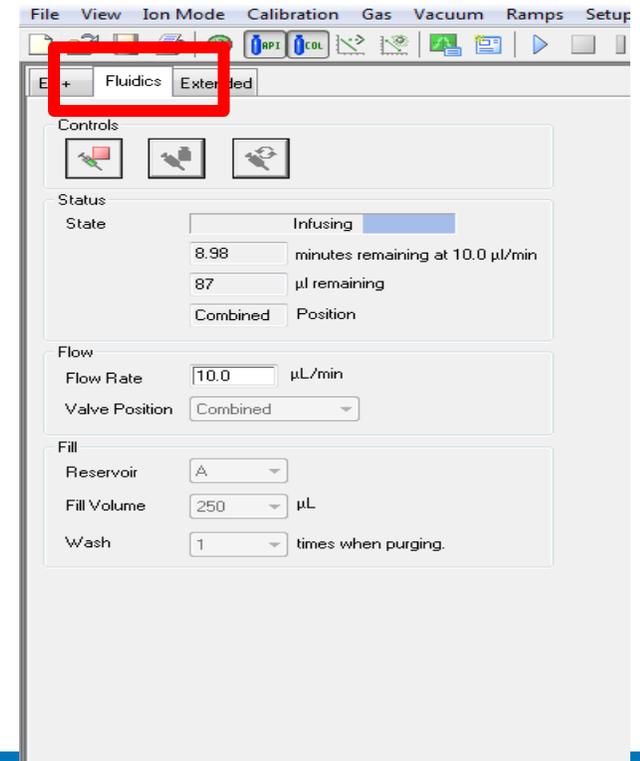
| Function | Set | Mass | Span | Gain |
|---------------|-------|---------|------|-------|
| MS1 Scan | 56 | 401 | 5 | 4 |
| MS1 Scan | 383.3 | 159 | 5 | |
| Daughter Scan | 401.3 | 159 | 5 | 1000 |
| MS1 Scan | 614 | 1821.95 | 2 | 32.49 |

Mass spectrum plot showing intensity vs m/z. The x-axis ranges from 385 to 403.5. Two major peaks are visible at approximately m/z 401 and 402.5. The peak at m/z 401 is significantly higher than the one at 402.5. The plot is zoomed in on the precursor mass region.

2

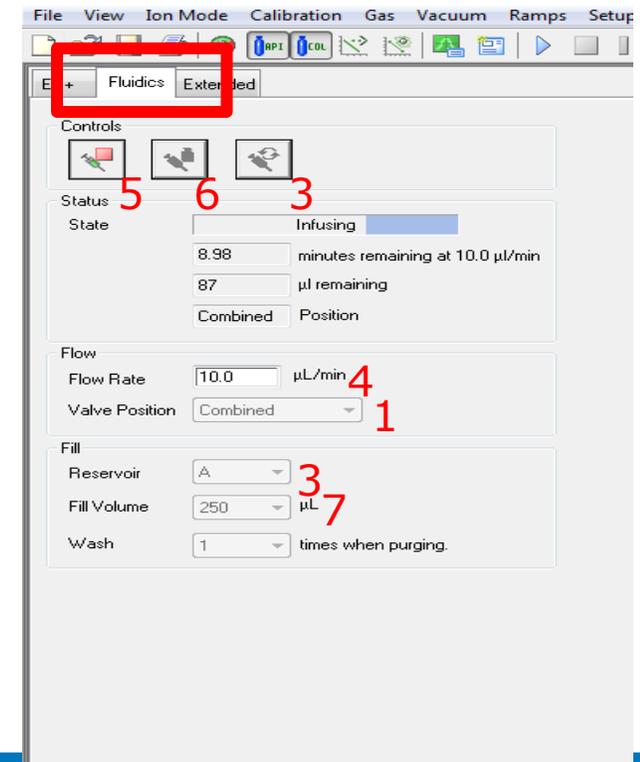
Fluidics Tab: preparation

- 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 guarantee 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 $\mu\text{L}/\text{min}$ (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 $\mu\text{L}/\text{min}$**
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**

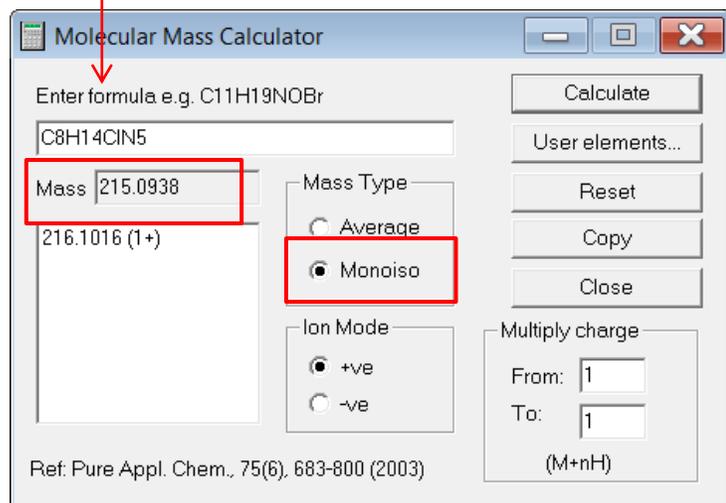


2. Run the IntelliStart process

- **Ionisation**
- **IntelliStart wizard**
- **IntelliStart report**

- 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 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.



http://www.chemspider.com/Chemical-Structure.2169.html?rid=b2e89b62-fac7-43d1-b6a3-aaa

File Edit View Favorites Tools Help

Home About us Web APIs Help Sign in

ChemSpider

Search and share chemistry

Simple Structure Advanced History

Found 1 result

Search term: **atrazine** (Found by approved synonym)

Atrazine

Molecular Formula C₈H₁₄ClN₅

Average mass 215.683 Da

Monoisotopic mass 215.093781 Da

ChemSpider ID 2169

herbicide pesticide

- 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:
 - $H_2O = M-18$
 - $MeOH = M-32$
 - $EtOH = M-46$

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.

IntelliStart Setup Parameters

Sample Tune and Develop Method

Compound Details

| Compound Name | Molecular Mass/Formula | Adduct A+ | Adduct B+ | Adduct A- | Adduct B- |
|--|------------------------|--------------------|-----------|-----------|-----------|
| <input checked="" type="checkbox"/> test | 603.3 | [M+H] ⁺ | | | |
| <input type="checkbox"/> | | | | | |
| <input type="checkbox"/> | | | | | |
| <input type="checkbox"/> | | | | | |

Multiply Charged Parents

Method Details

Create New Sample Tune Load Existing Sample Tune

Sample Tune Name: C:\MassLynx\Default.pro\Acqddb\Default.ipr

Develop SIR method: C:\MassLynx\Default.pro\Acqddb\Default.exp

Develop MRM method: C:\MassLynx\Default.pro\Acqddb\Default.exp

Keep HTML Name: Report

Invoke Manual Optimisation

Export To LC/MS System Check

Append to existing methods

Print to Printer Print to PDF

Optimization Ranges

Cone Voltage: Default (2 - 100) V

Collision Energy: Default (2 - 80) V

Daughter ion settings

Number of MRM transitions per compound: 5

Lowest Fragment Ion Mass: 50.0 Da

Exclude Losses...

Fluidics

Flow Path: Combined

Sample Reservoir: B

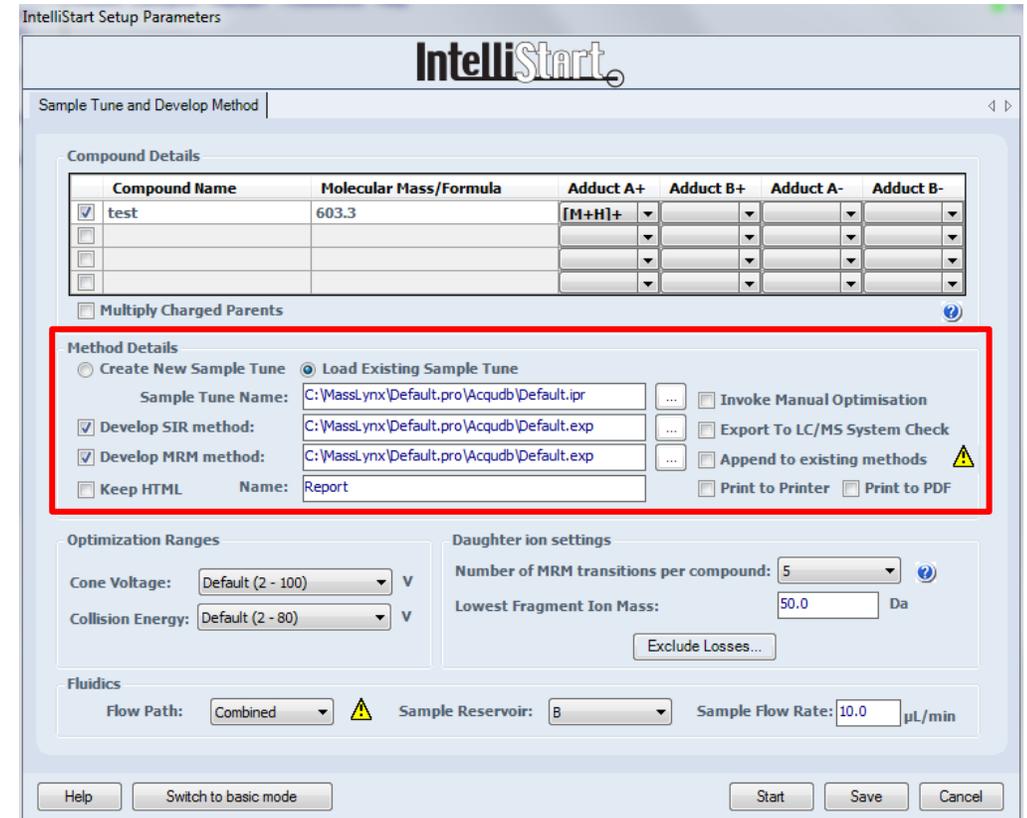
Sample Flow Rate: 10.0 µL/min

Help Switch to basic mode Start Save Cancel

1

2. Method Details – recommended settings:

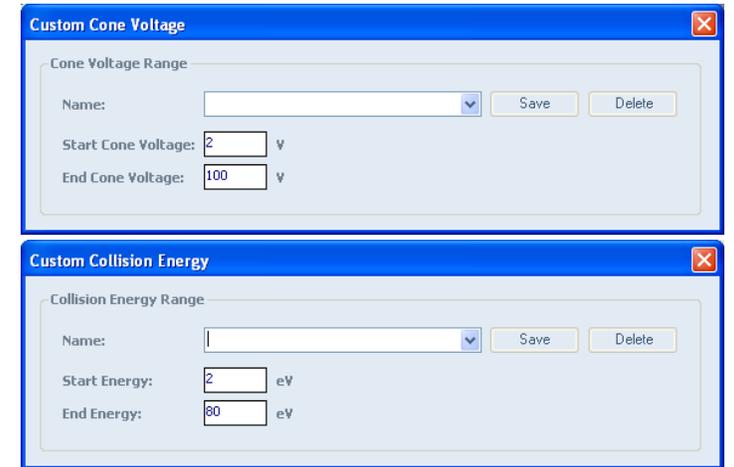
- Use the “Load Existing Sample Tune”: IntelliStart will use the information from this MS tune file directly and create an MS method with transitions, optimized cone voltages/collision energies. To load an existing tune page, of course a tune page needs to be created first. Apply the proper Resolution 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;



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.

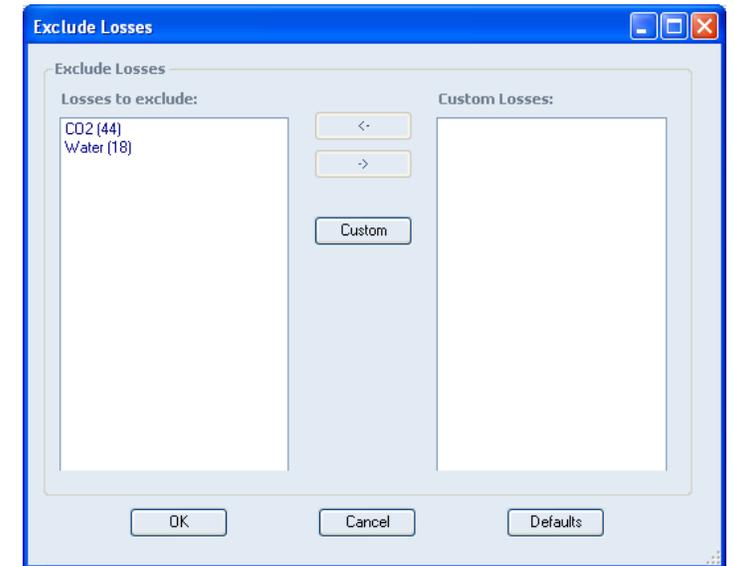
3



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



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

IntelliStart Setup Parameters

Sample Tune and Develop Method

Compound Details

| Compound Name | Molecular Mass/Formula | Adduct A+ | Adduct B+ | Adduct A- | Adduct B- |
|--|------------------------|--------------------|-----------|-----------|-----------|
| <input checked="" type="checkbox"/> test | 603.3 | [M+H] ⁺ | | | |
| <input type="checkbox"/> | | | | | |
| <input type="checkbox"/> | | | | | |
| <input type="checkbox"/> | | | | | |

Multiply Charged Parents

Method Details

Create New Sample Tune Load Existing Sample Tune

Sample Tune Name: C:\MassLynx\Default.pro\Acqddb\Default.ipr

Develop SIR method: C:\MassLynx\Default.pro\Acqddb\Default.exp

Develop MRM method: C:\MassLynx\Default.pro\Acqddb\Default.exp

Keep HTML Name: Report

Optimization Ranges

Cone Voltage: Default (2 - 100) v

Collision Energy: Default (2 - 80) v

Daughter ion settings

Number of MRM transitions per compound: 5

Lowest Fragment Ion Mass: 50.0 Da

Fluidics

Flow Path: Combined Sample Reservoir: B Sample Flow Rate: 10.0 µL/min

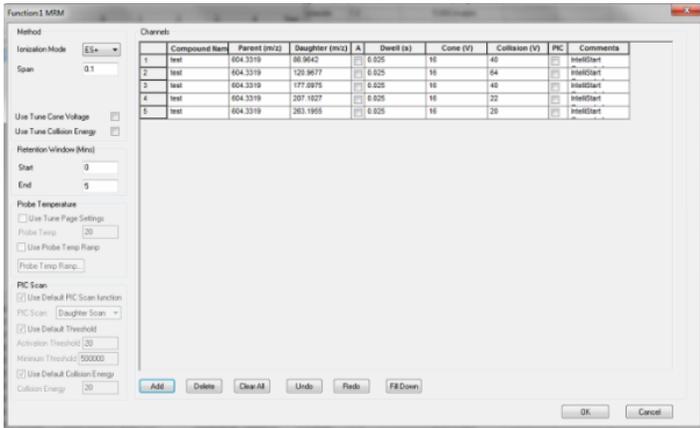
Start Save Cancel

5

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)



Method Development Report

| | |
|------------------------|---|
| MS1 Resolution | 0.75 |
| MS2 Resolution | 0.75 |
| Calibration Method | C:\MassLynx\IntelliStart\Results\Unit Resolution\Calibration_20190122_1.cal |
| Tune Method | C:\Masslynx\Projects\2019 Ring Acrylamide.PRO\ACQUDBtest.ipr |
| Cone Voltage Range | 2 - 100 |
| Collision Energy Range | 2 - 80 |
| Lowest Fragment Mass | 50.00 |
| Excluded Losses | 18.00, 44.00 |

Date: Generated on Wed 23 Jan 2019 at 13:56

Results

IntelliStart generated the following experiments:

MRM Experiment C:\Masslynx\Projects\2019 Ring Acrylamide.PRO\ACQUDBtest2.exp

IntelliStart found the following compounds:

| Compound | Formula/Mass | Parent m/z | Cone Voltage | Daughters | Collision Energy | Ion Mode |
|----------|--------------|------------|--------------|-----------|------------------|----------|
| test | 603.3 | 1 604.33 | 16 | 177.10 | 36 | ES+ |
| | | 2 604.33 | 16 | 263.20 | 22 | ES+ |
| | | 3 604.33 | 16 | 207.10 | 20 | ES+ |
| | | 4 604.33 | 16 | 86.96 | 34 | ES+ |
| | | 5 604.33 | 16 | 120.97 | 50 | ES+ |

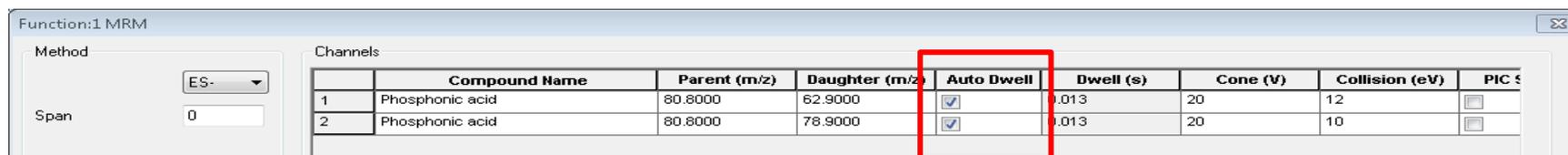
Compound
test

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.

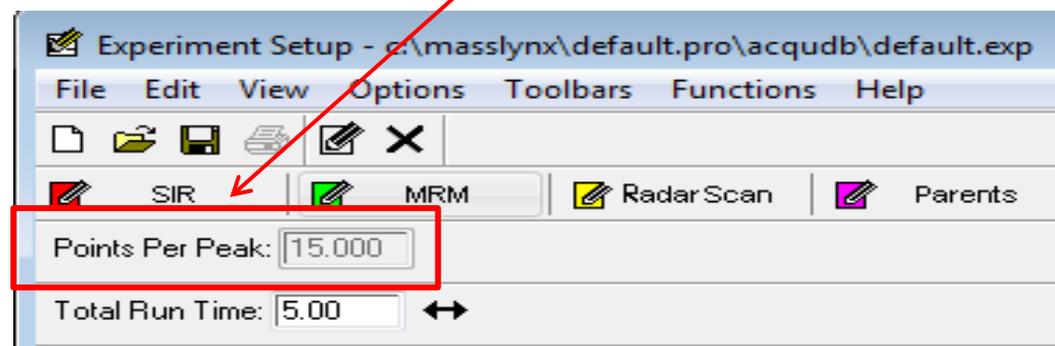
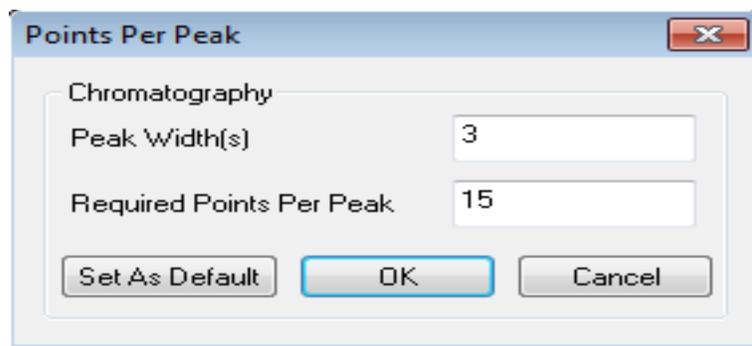
Additional remarks regarding the MS Method

- 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.

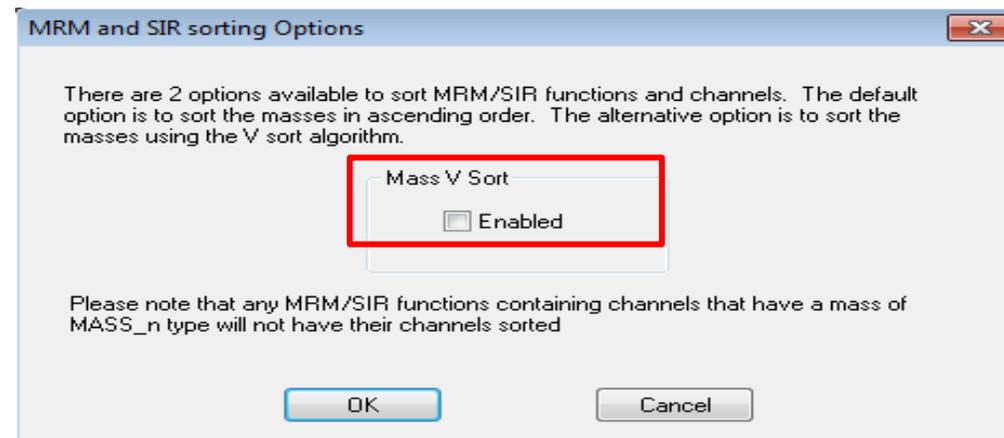


| | Compound Name | Parent (m/z) | Daughter (m/z) | Auto Dwell | Dwell (s) | Cone (V) | Collision (eV) | PIC |
|---|-----------------|--------------|----------------|-------------------------------------|-----------|----------|----------------|--------------------------|
| 1 | Phosphonic acid | 80.8000 | 62.9000 | <input checked="" type="checkbox"/> | 0.013 | 20 | 12 | <input type="checkbox"/> |
| 2 | Phosphonic acid | 80.8000 | 78.9000 | <input checked="" type="checkbox"/> | 0.013 | 20 | 10 | <input type="checkbox"/> |

- 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.



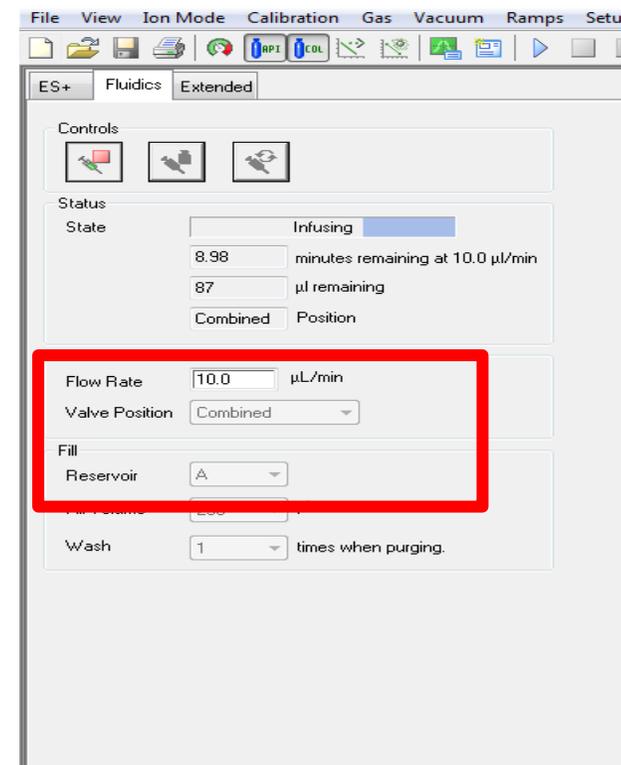
- There are two options for sorting out the MRM transitions:
 1. acquiring masses in ascending order
 2. V-sort algorithm (acquired in the optimum order of performance, minimizing large voltage jumps)
- This can be important when very short dwell times and inter-scan delay times are used. It is recommended 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**

- 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

ES- Source | Fluidics | Advanced | Diagnostics

Source Fitted: ESI:1

Voltages

Capillary (kV): 2.45 | 2.40

Cone (V): -82 | 25

Temperatures

Desolvation Temp (°C): 650 | 650

Gas Flow

Desolvation (L/Hr): 993 | 1000

Cone (L/hr): 295 | 300

Nebuliser (Bar): 6.6 | 7.0

Analyser

LM Resolution 1: 3.00

HM Resolution 1: 15.00

Ion Energy 1: 1.0

LM Resolution 2: 2.70

HM Resolution 2: 15.00

Ion Energy 2: 2.0

Collision Gas Flow (mL/Min): 0.19 | 0.20

Collision: 2

| Function | Set | Mass | Span | Gain |
|-----------------|-------|---------|------|-------|
| 1 MS1 Scan | 56 | 401 | 5 | 4 |
| 2 Daughter Scan | 383.3 | 159 | 5 | 1000 |
| 3 Daughter Scan | 401.3 | 159 | 5 | 1000 |
| 4 MS1 Scan | 614 | 1821.95 | 2 | 32.49 |

3.08e7 401.0

8.5 399.0 399.5 400.0 400.5 401.0 401.5 402.0 402.5 403.0 403.5

Pumped Operate SWave Optimisation file

1